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AN INVESTIGATION INTO THE  
BIOSYNTHESIS OF TERREIN

BY

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A thesis presented for part fulfillment of the  
requirements for the Degree of Doctor of Philosophy

Department of Chemistry  
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March 1992

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## DEDICATION

This thesis is dedicated to the memory  
of my father Michael McCusker.

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## Summary.

An investigation has been carried out into the nature of the biosynthesis and the metabolites involved in the production of the natural product, terrein. Terrein, a metabolite of the fungus Aspergillus terreus has been previously shown to be of polyketide origin. It seems likely that terrein may be derived by way of an aromatic intermediate with subsequent oxidative cleavage of the aromatic ring to a five-membered ring. It is the nature of this ring contraction which is of primary interest. Also under investigation was the stereochemistry of the elimination process leading to the propenyl side chain of terrein.

3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin is already known to be an intermediate on the pathway to terrein and this fact was utilised in the investigation of the side chain modification of terrein. The two deuterium labelled isocoumarins [ $4, \alpha, \alpha, \alpha$ - $^2\text{H}_4$ ]-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin and [ $3,4$ - $^2\text{H}_2$ ]-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin were synthesised and fed to Aspergillus terreus. Results showed that both were incorporated to some extent but some partial degradation of the isocoumarins to acetate was also observed.

In the ring contraction step it is known that C-7 of the isocoumarin is lost. Thus a possible intermediate containing an oxygenated carbon at C-7 was



examined. Several synthetic routes to 3,4-dihydro-6,7,8-trihydroxy-3-methylisocoumarin were investigated since the synthesis used for the 6,8-dihydroxyisocoumarin was not applicable in this case. The intermediacy of 2,3,5-trihydroxyphenylpropene was also explored. Two different synthetic strategies to this compound were employed, one involving the condensation of a phosphonic acid bisamide with an appropriate ester derivative, the other making use of the ortho-Claisen rearrangement.

## Chapter 1.

### Introduction.

The chemistry of natural products has been studied seriously for about one hundred and fifty years<sup>1</sup>, but investigation of their biosynthesis is a much more recent endeavour. This has been greatly facilitated by the advent of modern spectroscopic techniques and the availability of isotopically labelled compounds. The term "natural product" is commonly reserved for those organic compounds of natural origin that are unique to one organism. In most instances they appear to be non-essential to the plant, insect or microorganism producing them. The chemist studying natural products depends heavily on the type of living organism chosen for his biogenetic research. It is therefore important that one should understand the essential biological and taxonomic features of the organism which is under study.

There are about two billion different kinds of living things on the earth, of which fungi make up approximately one hundred thousand species.<sup>2</sup> Many of these fungi are so prevalent and abundant that they must be considered one of the more successful forms of life. Laymen are generally familiar with many fungi such as mushrooms or toadstools in forests and fields, bracket fungi on trees and perhaps puffballs which send forth clouds of spores when kicked. The housewife uses yeast to leaven her bread and the brewer uses the same organism to form alcohol in his beer. Consequently, fungi have been

found to be of basic and practical significance in various fields, from human medicine to grain storage, from agriculture to architecture, from fundamental studies of the nature of sex to biological warfare. There are few phases of our life, apparently, into which fungi do not enter, for good or bad.

The fungi along with the algae and bacteria are members of the Thallophyta, a somewhat artificial division of the plant kingdom comprising of organisms with no true roots, stems or leaves. The fungi as a group share three main characteristics:-

1. They have no chlorophyll. This means that they cannot manufacture their own organic food such as sugars, starches, cellulose etc, as the green plants do. They consequently have to live on the remains of other plants or animals or on living plants and animals, in other words they must live as saprophytes or parasites.
2. The growing or food-getting part of the fungus is made up of long hollow branched cells known as hyphae, which in aggregate are called mycellium.
3. Fungi reproduce by means of spores which makes for rapid increase, far travel and wide distribution. There is almost no other form of life that can equal them in these capacities.

The classification of fungi rests upon the type of

spores which are formed, together with the nature of the mycelium.<sup>3,4</sup> The class within which our interest lies are known as the Fungi Imperfecti; that is those organisms for which no sexual (perfect) stage has been observed. Members of this class form asexual spores borne on conidiophores which usually occur on the surface of the mycelium. The most common genera are the Penicillia and the Aspergilli which are often responsible for the grey-green growth on "mouldy" food. The genus Aspergillus is a very large one deriving its name from "aspergillum" meaning a mop, which aptly describes the conidial heads. The majority of the seventy-eight species recognised by Thom and Raper are known only in the conidial stage. It is this genus that the forthcoming chapters will be mainly concerned with.

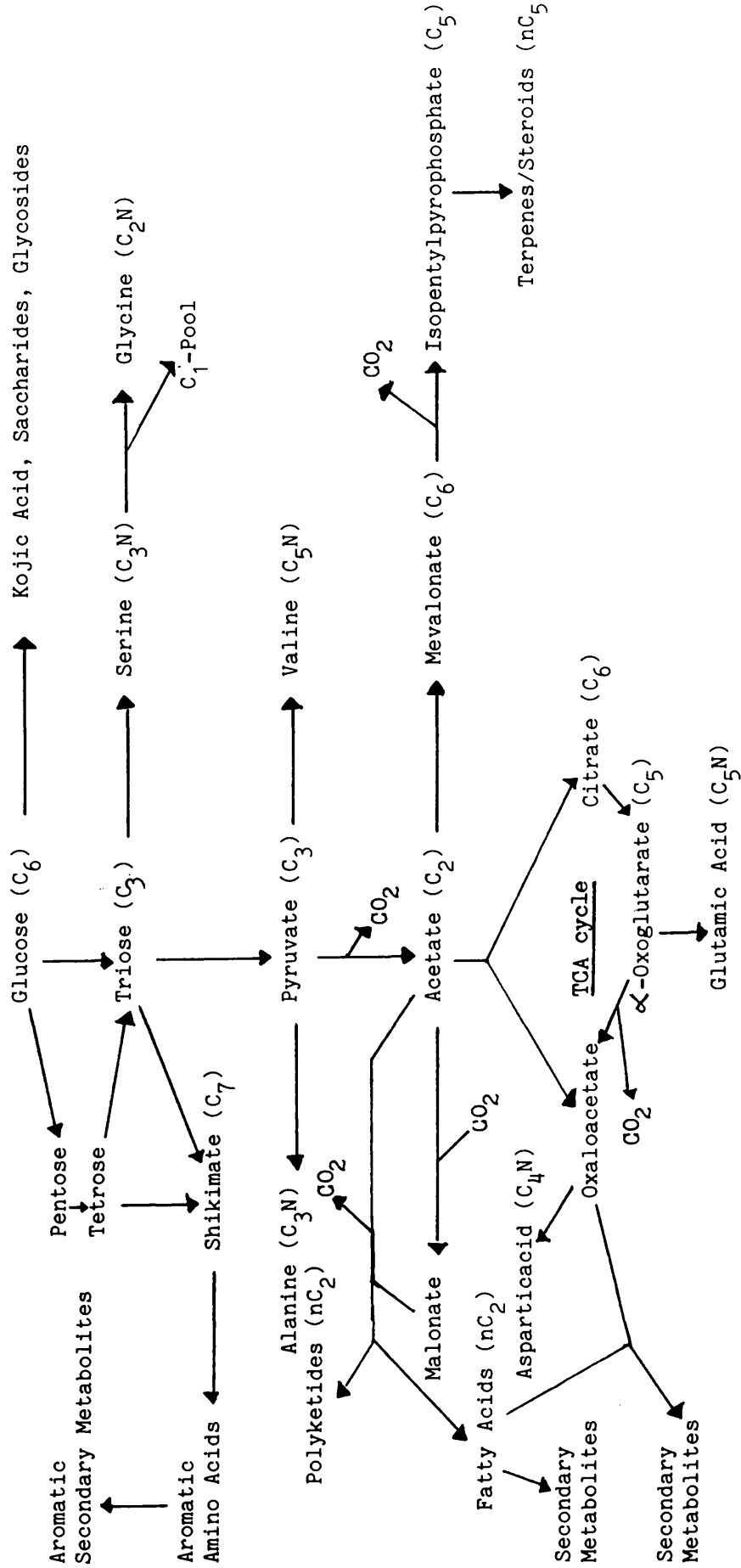
Fungi are among the most active of chemists and are able to produce a large variety of organic compounds. Many of these compounds are of no apparent value to the fungus as they are not directly involved in supplying energy to the cell. These fungal products are thus known as secondary metabolites, so called because they play no obvious role in the metabolism of the organisms which produce them. The great advantage of fungi as sources of secondary metabolites is their ability to produce the compounds on aqueous media. As a result, secondary metabolites of diverse type are conveniently available in the laboratory for chemical, biochemical and biological studies.

The dividing line between primary and secondary metabolism is rather blurred<sup>1</sup>: there are many obscure amino acids that are definitely secondary metabolites, while many steroid alcohols have an essential structural role in most organisms and must therefore be considered primary metabolites. In addition, the two types of metabolism are interconnected, since primary metabolism provides a number of small molecules which are often employed as starting materials for all the important secondary metabolic pathways. (Scheme 1).

There are three principal starting materials for secondary metabolism:

- (i) SHIKIMIC ACID, the precursor of many aromatic compounds including the aromatic amino acids, cinnamic acids and certain polyphenols.
- (ii) AMINO ACIDS, leading to alkaloids and peptide antibiotics including the penicillins and cephalosporins.
- (iii) ACETATE, precursor of polyacetylenes, prostaglandins, macrocyclic antibiotics, polyphenols and the isoprenoids. It also plays a major role in the biosynthesis of fatty acids and polyketides.

Our discussion will be limited to the role of acetate in secondary metabolism since polyketides are the characteristic secondary metabolites of the fungi imperfecti. The polyketide route leads almost exclusively to secondary metabolites and is used mainly by fungi and



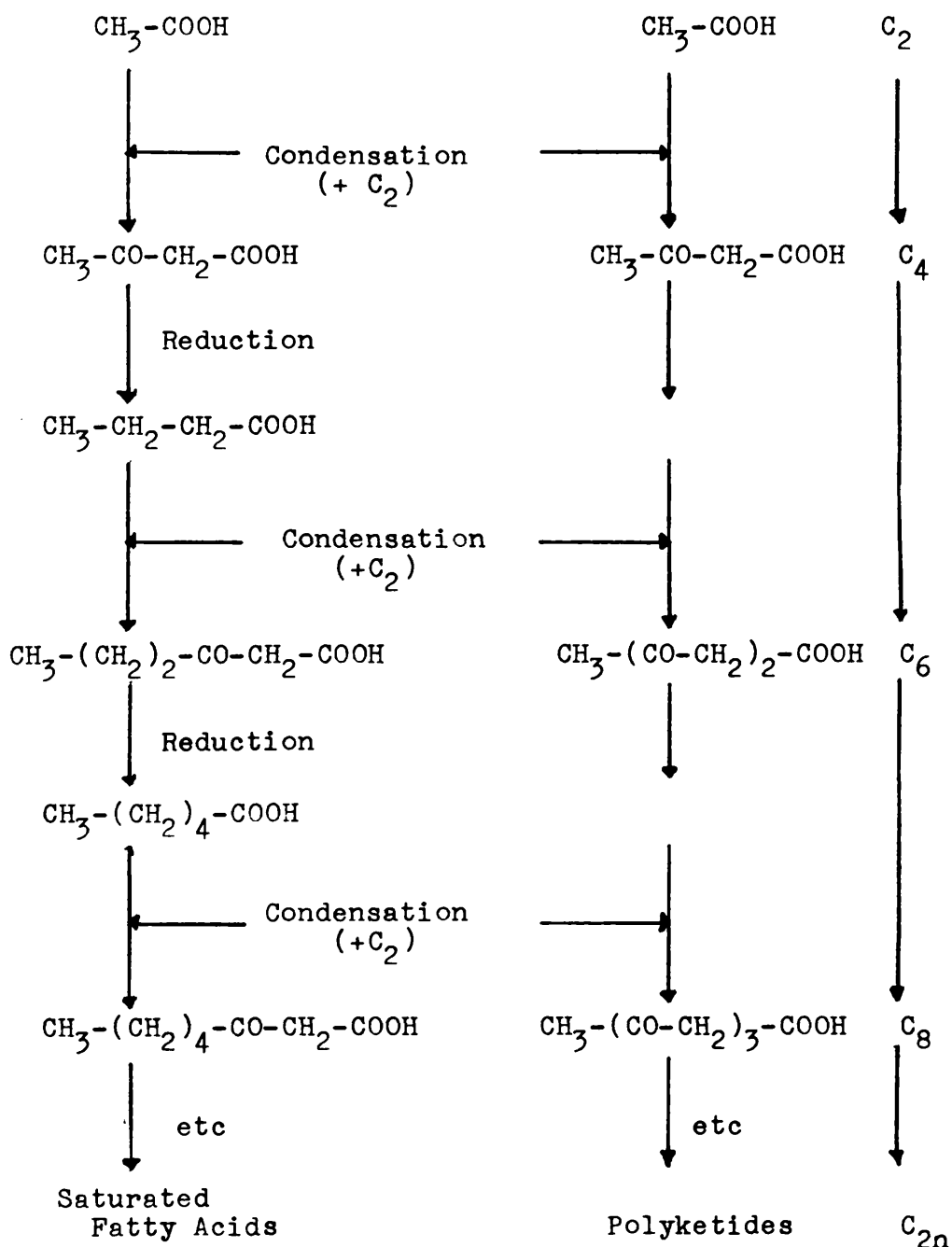
Scheme 1.

to a lesser extent by bacteria and higher plants. Even among the fungi, the ability to produce polyketides is not evenly distributed. Consequently, because of the limited distribution and secondary nature of polyketides, the concepts of polyketide biosynthesis were developed by organic chemists but the problem has received little attention from biochemists.<sup>5</sup> It is for this reason that although the basic tenets of the hypothesis have been firmly established by labelling experiments, there is less known about the detailed mechanisms of polyketide biosynthesis than any other biosynthetic pathway.

The  $C_2$ - unit of acetate is one of the most common "building blocks" used by living organisms for generating complex molecules. Acetic acid in its activated forms of acetyl CoA and malonyl CoA are two of the most vitally important carbon sources, being used to generate terpenes, fatty acids and the compounds we are most concerned with, polyketides.

Polyketides arise from the condensation of an acetyl unit, or other acyl units, with malonyl units to produce polyketomethylene chains,<sup>4</sup>  $[-(CH_2-CO)_n-]$ . There is a close parallel between the biosynthesis of fatty acids and the biosynthesis of polyketides, since in both cases the formation of linear chains proceeds by the addition of  $C_2$ -units. However, whilst in fatty acid biosynthesis every  $C_2$ - unit is added to the growing chain only after reduction of the previous carbonyl unit to a methylene group, the growth of the polyketide chain does not normally require

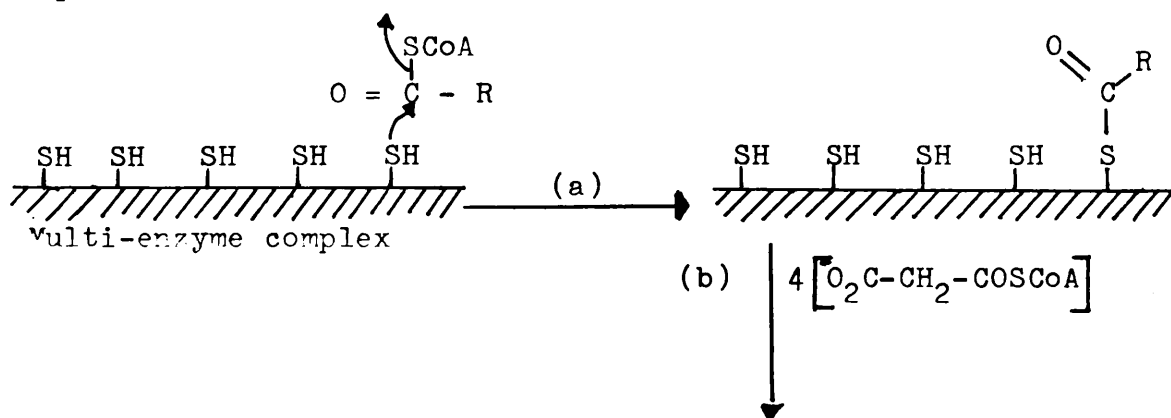
such an obligatory prior reduction. Thus linear polyketoesters of varying chain lengths may be formed depending on the number of acetate and malonate units involved. The major difference in the assembly of acetate units during the biosynthesis of fatty acids and polyketides is shown in Scheme 2.

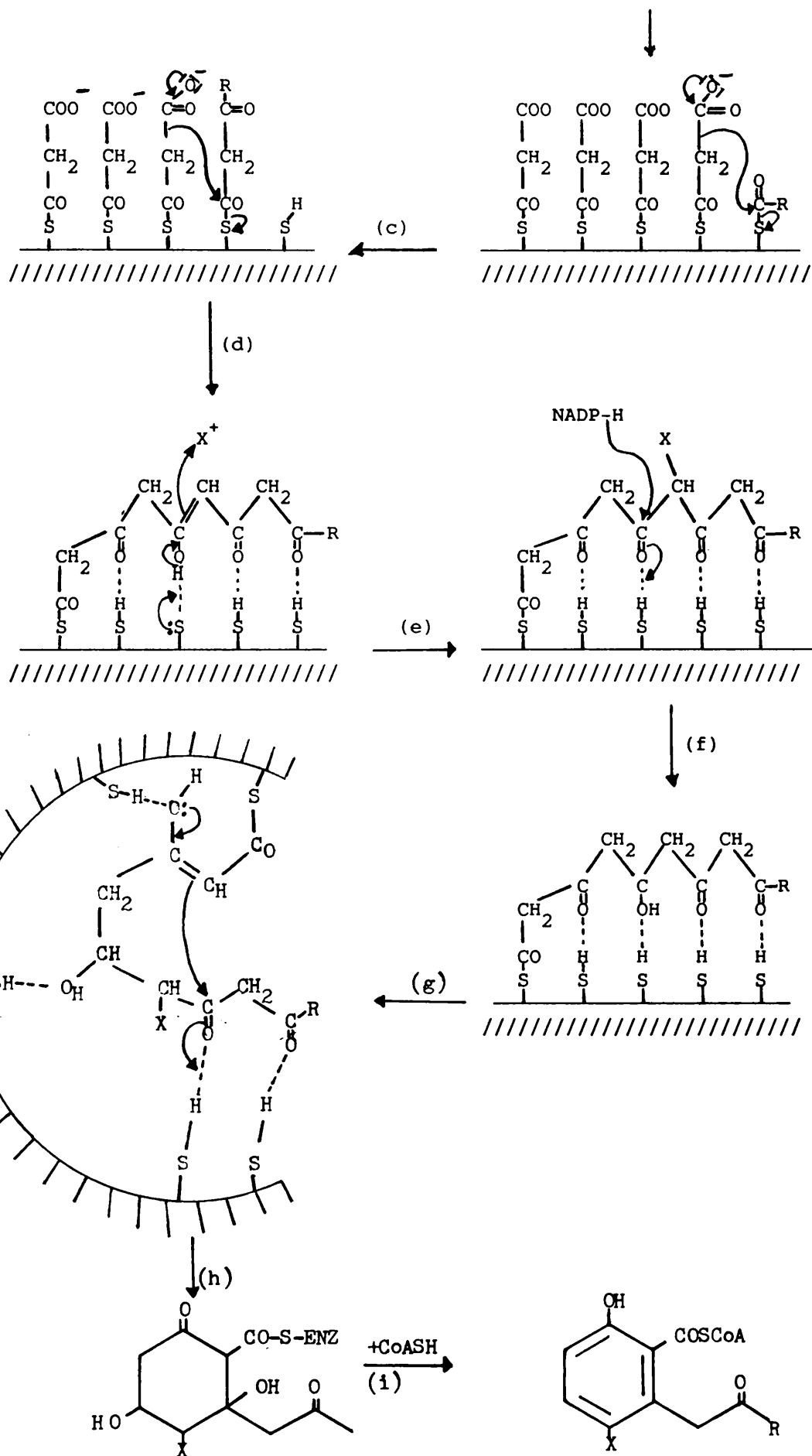


Scheme 2.



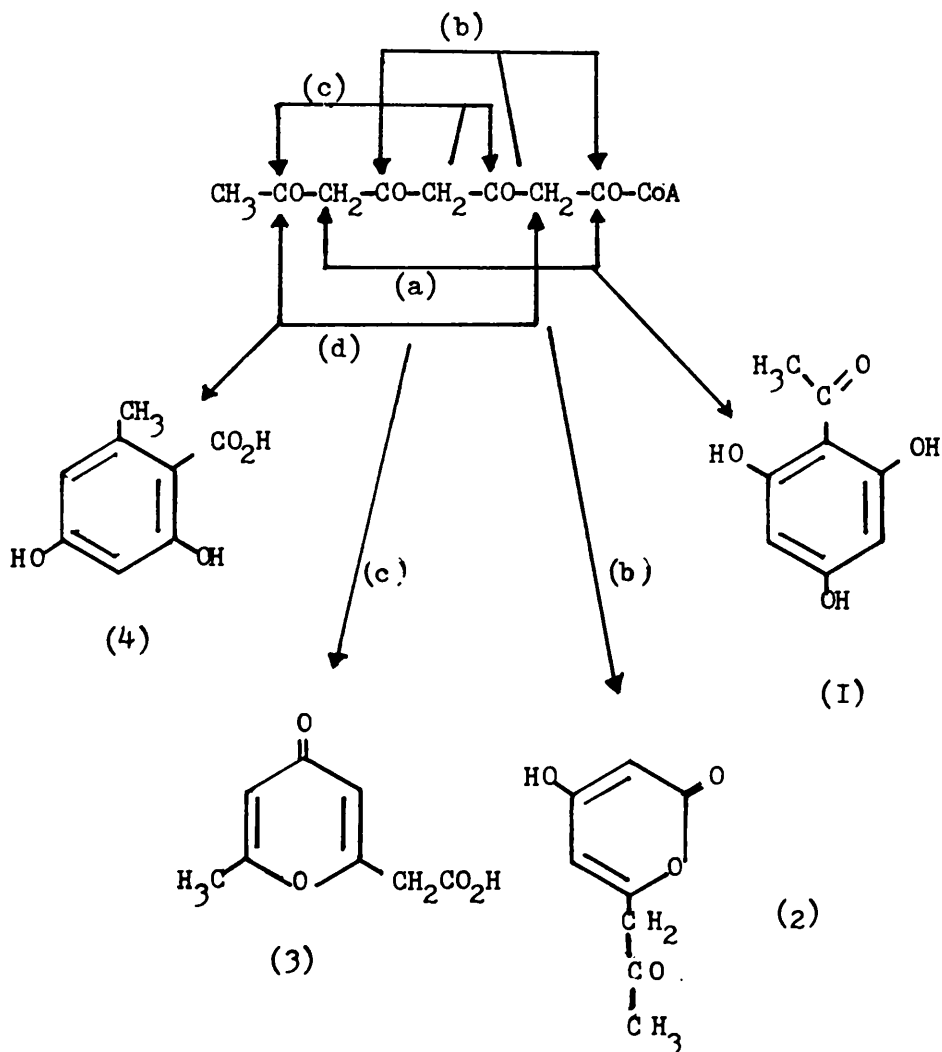
The  $\beta$ -ketoesters formed from the condensation of acetyl and malonyl units are known to be very reactive compounds. This is due to the fact that they possess activated methylene groups (potential nucleophiles) and carbonyl groups (potential electrophiles) which can take part in internal Claisen or Aldol-type condensations to give aromatic type compounds which are susceptible to electrophillic substitution. The  $\beta$ -polyketoester (polyketide) is temporarily stabilised by chelation or hydrogen bonding on the enzyme surface until assembly is accomplished.<sup>6</sup> The cyclisation is then guided by the special topology of the enzyme. The activated methylene groups give rise to carbanions or enolates by removal of protons and the polarised carbonyl group has carbonium character. A possible model for the enzyme system involved in the biosynthesis of polyketides can be seen in Scheme 3. It is thought that an acyl group bound to a thiol group of a multienzyme complex, reacts with a malonyl unit bound to the complex bound by a second sulphur bridge which is situated in an appropriate position.





**SCHEME 3**

As mentioned before, intramolecular reactions, particularly Aldol and Claisen are quite a common occurrence in linear polyketomethylinic chains. In the simplest of cases a polyphenolic aromatic nucleus is produced by cyclisation of these chains. The benzene nucleus may be formed via several paths. For example, in Scheme 4 path (a) leads to the acetophenone derivative xanthoxylin (1), paths (b) and (c) to the pyrone derivatives (2) and (3) and path (d) to orsellinic acid (4), all of which are known natural products.



Scheme 4

A wide variety of metabolites may be formed in polyketide biosynthesis depending upon a number of different factors such as:-

1. The starter unit or chain initiating unit.
2. The number of acetyl CoA units involved, or occasionally other esters such as propionyl or butyryl CoA.
3. The mode of cyclisation.
4. The condensation of separately synthesised polyketides.
5. The secondary processes such as halogenation, alkylation, rearrangements, redox reactions etc.

It was as early as 1907 that Collie<sup>9</sup> demonstrated the in vitro cyclisation of polyketides, and suggested that a similar process may occur in vivo. Collie's idea was not followed up until 1953 when Birch rediscovered it and proposed it as a general biogenetic hypothesis to account for the structure of a large number of natural products. Birch's biogenetic acetate rule states that the synthesis of polyketides usually takes place in four successive steps.

- (i) The head to tail addition of  $C_2$ -acetate units (Chain assembly).
- (ii) Oxidations, reductions and alkylations of the polyketide chain.
- (iii) Stabilisation of these chains via intramolecular cyclisations.

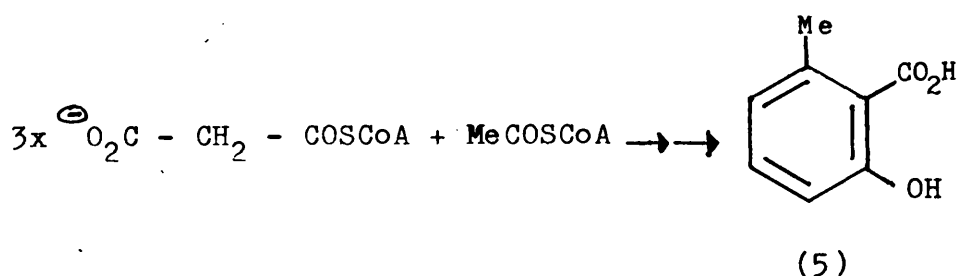
(iv) Secondary modifications of the functions or of the mono or polycyclic skeleton resulting from the previous step.

Steps (ii) and (iv) do not always occur; for certain biosyntheses both "one chain" and "two chain" pathways can be formulated but, whenever such cases have been tested experimentally, the single chain process has almost always been found. It is therefore generally accepted that the biosynthesis of polyketides normally involves only one polyketide chain.

Birch established via a series of elegant experiments that a wide range of structural types were derived from acetate and malonate. Support for the acetate hypothesis was first confirmed by an investigation of the labelling pattern of 6-methylsalicylic acid (5) produced by Penicillium griseofulvum. Birch showed that  $[1-^{14}\text{C}]$ -acetate was incorporated into (5) and degradation experiments showed a labelling pattern which was consistent with the hypothesis. Kuhn-Roth degradation showed that the acetic acid produced, contained a label at the carboxyl carbon thus suggesting that it is derived from C-6 of 6-methylsalicylic acid. Decarboxylation of (5) produced carbon dioxide which contained a label, while the bromopicrin derivative (6) was found to be unlabelled (Scheme 5).

To establish the starter unit,  $[2-^{14}\text{C}]$  malonate was fed to the fungus and 6-methylsalicylic acid was isolated and analysed. As expected the  $\text{C}_2$ -unit derived from

acetate contained very little activity. In contrast, when  $[2-^{14}\text{C}]$  acetate was fed the methyl carbon was found to contain a label.



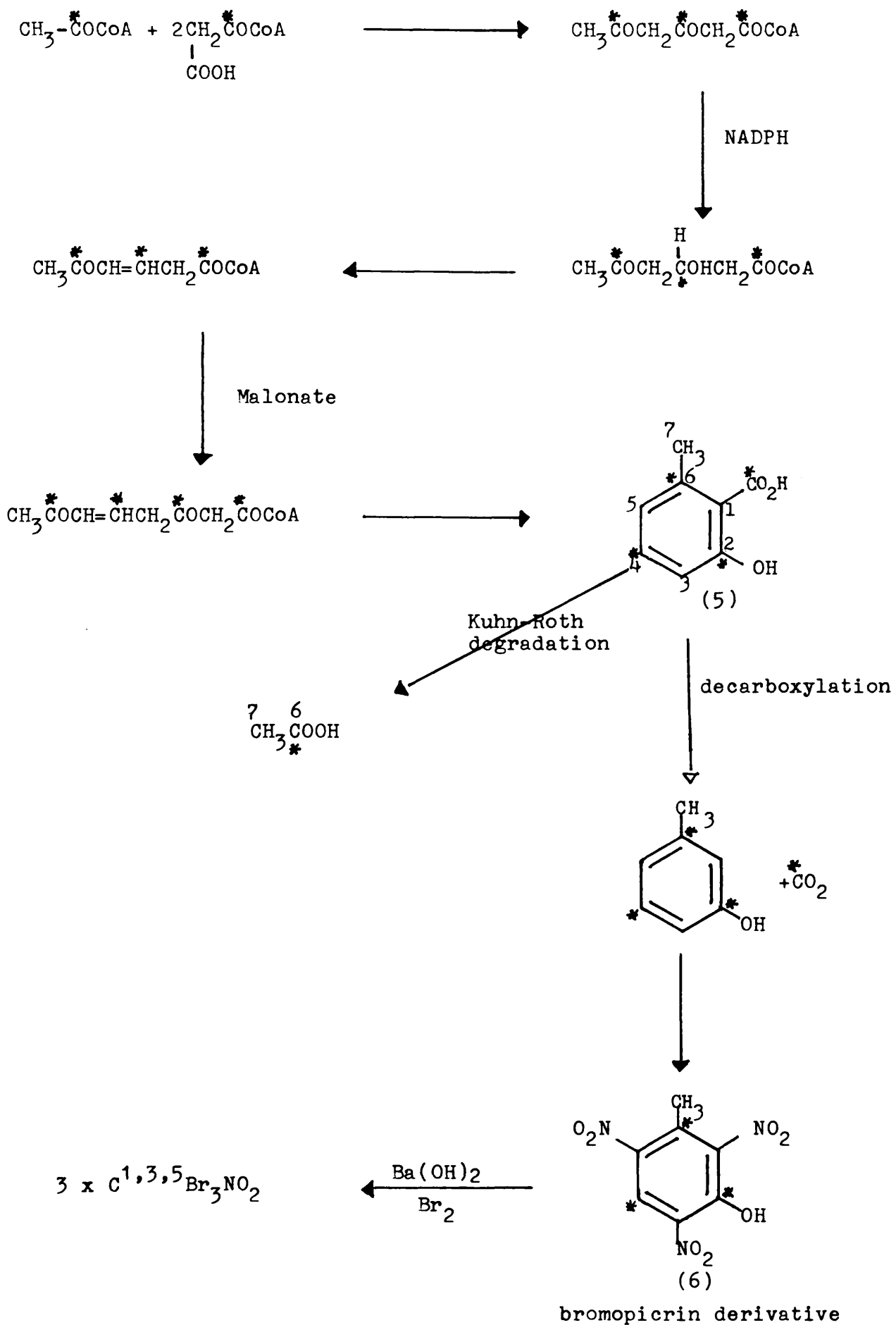
$^{18}\text{O}$ -labelled acetic acid was also fed to *P. griseofulvum*. The 6-methylsalicylic acid isolated was shown to contain  $^{18}\text{O}$ -labelled oxygens thus proving that the original oxygens are retained throughout the biosynthesis. This being in complete agreement with the acetate hypothesis.

### Modification of Polyketide Chains

Structural variety can be introduced into polyketide derived molecules in a variety of ways. Such reactions may take place when the polyketide product has either formed or is in the process of formation. In most cases the exact point where such modifications take place has not been determined.

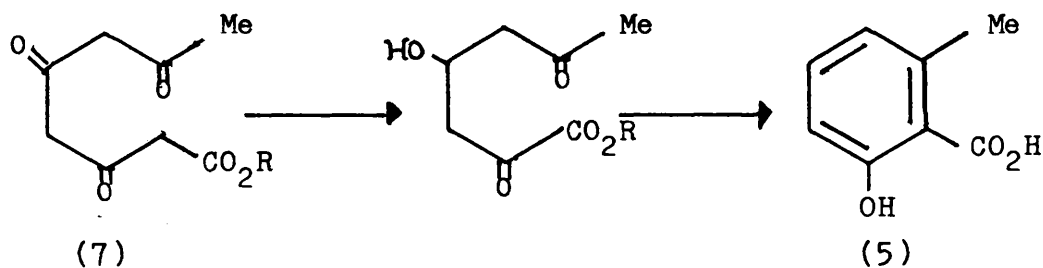
#### A. Loss of Oxygen

Loss of one or more oxygen atoms from the polyketide chain could be achieved by reduction of the ketone and



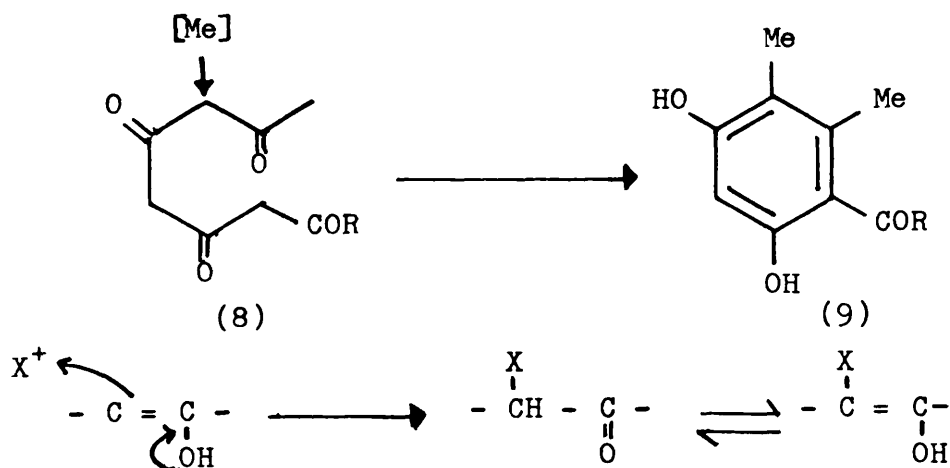
Scheme 5.

dehydration of the resulting alcohol as in fatty acid biosynthesis. 6-Methylsalicylic acid (5) can arise from the orsellinic acid precursor (7) by such a process. In other metabolites the dehydration may be followed by reduction of the double bond to give saturated systems.



#### B. Introduction of Alkyl Groups

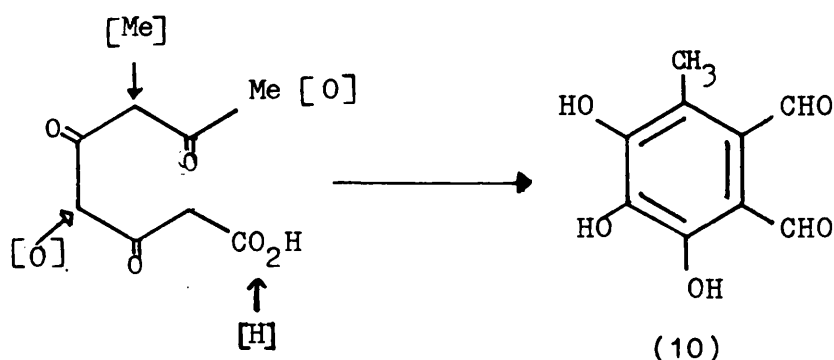
The introduction of alkyl groups can occur electrophilically, eg. methyl from methionine and isoprenoid units from their pyrophosphates. 4,6-Dihydroxy-2,3-dimethyl benzoic acid (9) can be formed by methylation of the orsellinic acid precursor (8). C-Alkylation always occurs at positions corresponding to the methylene groups of the polyketide chain.





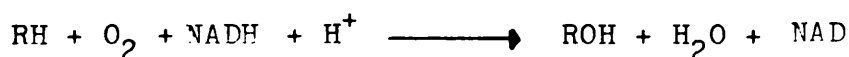
### C. Oxidation and Reduction

It is possible to oxidise alkyl groups and for carboxyl groups to be reduced. For example, in the biosynthesis of flaviphan (10), an extra methyl group is introduced, another methyl group is oxidised to an aldehyde and a carboxyl group is reduced to an aldehyde.



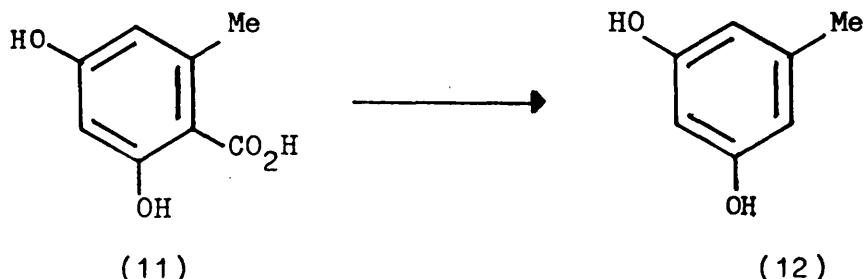
### D. Introduction of Oxygen

Flaviphan (10) above, illustrates a further common reaction of polyketide biosynthesis:- the introduction of extra oxygen atoms. This process is probably catalysed by a mixed function oxygenase, so called because they catalyse the introduction of one atom of an oxygen molecule into the substrate while at the same time the other is reduced to water. The hydrogen being derived ultimately from a carrier such as NADH.



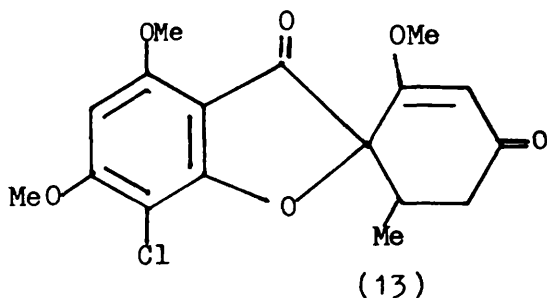
### E. Decarboxylation

This is a frequent reaction of polyketide derived molecules. It is illustrated in the formation of orcinol (12) from orsellinic acid (11) a constituent of many fungi and lichens. Coupled to the oxidation of methyl to carboxyl this can result in the loss of a methyl group.



### F. Inter and Intra-oxidative Coupling

These can occur with the formation of carbon-carbon or carbon-oxygen bonds. This is exemplified in the formation of griseofulvin (13)



Several groups of enzymes are capable of catalysing oxidative phenolic coupling. They have iron or copper as a prosthetic group and are all able to effect one electron transfers.

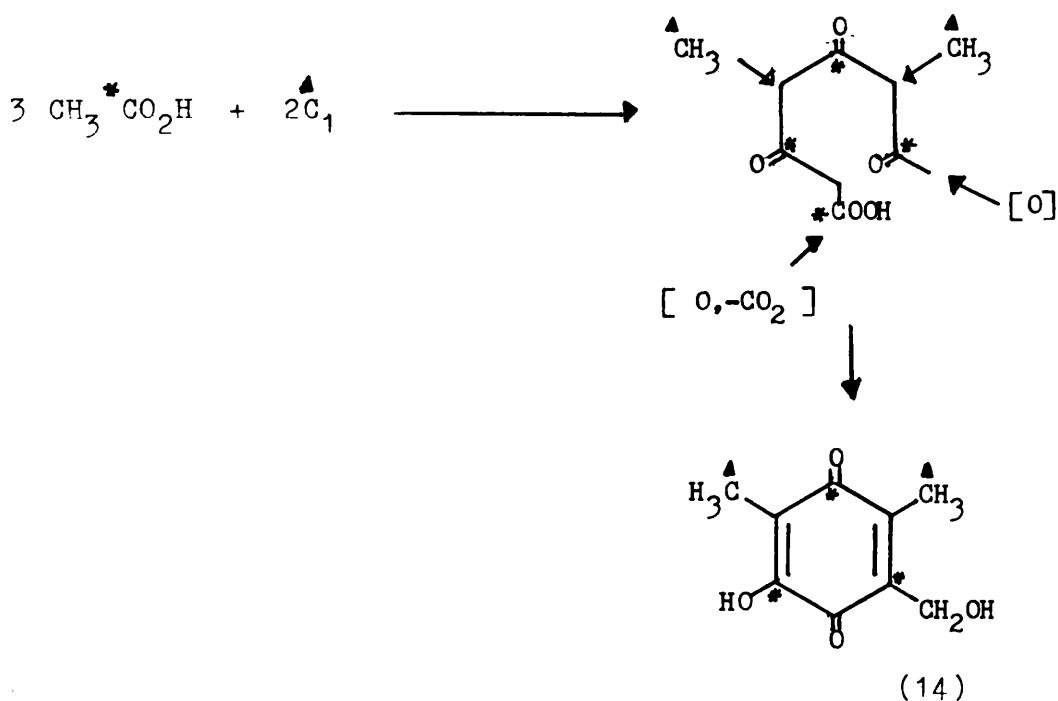
Nowadays modern structural analysis relies heavily on spectroscopic methods and tries more and more to steer clear of chemical reactions such as degradation experiments. The main reason for this being simply that the amount of material available is usually too small. The experimental study of biosynthesis has developed largely through the application of tracer studies using compounds which have been isotopically labelled with  $^{14}\text{C}$  or  $^3\text{H}$ . Adequate n.m.r. instrumentation for recording  $^2\text{H}$ -,  $^3\text{H}$ - and  $^{13}\text{C}$ -labelled compounds became available in the sixties, revolutionising biosynthetic studies. The great advantage of n.m.r. analysis is that degradation methods can virtually be totally avoided. The techniques of deuterium and carbon-13 n.m.r. are of particular relevance to the biosynthetic work discussed in this thesis. A short review of the main features of these two techniques will be discussed in the next few pages.

### Carbon-13 N.M.R.

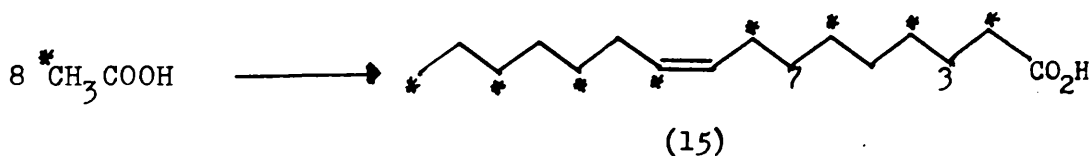
$^{13}\text{C}$  N.m.r. spectroscopy is the most important physical method for the investigation of biosynthetic pathways. Carbon-13 is stable, with a nuclear spin of  $1/2$  and a positive nuclear Overhauser effect. The isotope itself makes up only 11 per cent of naturally occurring carbon but the sensitivity of modern spectrometers make this level quite adequate for the measure of  $^{13}\text{C}$  n.m.r. spectra. Indeed, the low natural abundance is actually an advantage. Only occasionally is a  $^{13}\text{C}$  near enough to

another  $^{13}\text{C}$  for any  $^{13}\text{C}$ - $^{13}\text{C}$  spin-spin coupling to occur, the spectra do not normally show splitting from this cause and are thus enormously simplified. The  $^{13}\text{C}$  shift range (200 ppm) is considerably larger than  $^1\text{H}$  (10 ppm) and gives narrow line widths. The spectra are thus well resolved.  $^{13}\text{C}$  However has unfortunately a low sensitivity (one-sixtieth of  $^1\text{H}$ ), although this can in part be compensated for by proton decoupling. This causes the collapse of the multiplets to single sharp peaks and intensity enhancement due to the positive nuclear Overhauser effect. By proton noise decoupling, all  $^{13}\text{C}$ - $^1\text{H}$  coupling is lost and by off-resonance techniques residual couplings will give information on the number of hydrogens attached to the carbon. A quaternary carbon is seen as a singlet, a methylene carbon as a triplet, a methine carbon as a narrow doublet, and a methyl carbon as a quartet.  $^{13}\text{C}$  N.m.r. has been used with success in tracing the fate of hydrogen in biosynthesis.

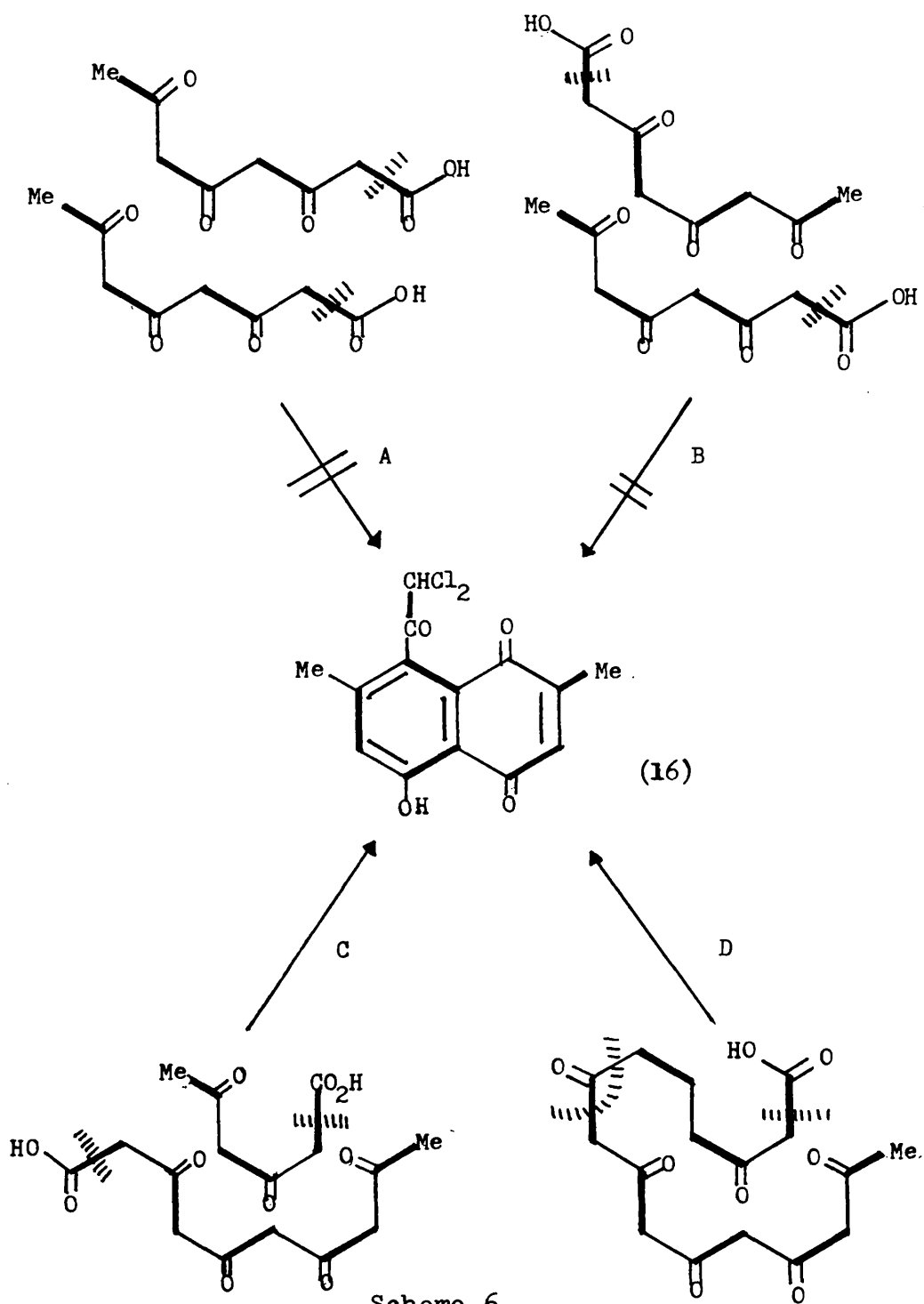
Shanorellin (14) is a benzoquinone pigment synthesised by Shanorella spirotricha. On administration of [ $1\text{-}^{13}\text{C}$ ]-acetate or  $^{13}\text{C}$ -formate to the culture medium,  $^{13}\text{C}$ -enriched shanorellins are obtained.



Palmitoleic acid (15) which is produced by the fungus Saccharomyces cerevisiae, when supplemented with [2-<sup>13</sup>C]-acetate gave a spectrum showing enhanced signal intensity for every second carbon atom in agreement with theory.<sup>12</sup> This experiment demonstrates the strength of the <sup>13</sup>C n.m.r. technique in that the very similar carbon atoms C<sup>3</sup>-C<sup>7</sup> appear well resolved in the <sup>13</sup>C n.m.r. spectrum of (15).



The use of doubly labelled acetate,  $[1,2-^{13}\text{C}]$ -acetate is a technique employed to give information on the number of intact acetate units in a molecule. In this way, all the carbon atoms of a molecule which remain intact are labelled but since adjacent  $^{13}\text{C}$ -nuclei exhibit C-C coupling some resonances will be split into doublets. By comparing the observed coupling constants the adjacent nuclei may be identified and can be assumed to be derived from an intact acetate unit. For example in biosynthetic studies with the natural product mollisin (16). The polyketide nature of mollisin, established by Bentley and Gatenbeck<sup>13</sup>, has been confirmed using  $[2-^{13}\text{C}]$  acetate. However the pattern of intact acetate units observed after incorporation of  $[1,2-^{13}\text{C}_2]$ acetate<sup>14,15</sup> excludes both of the tetraketide "two-chain" derivations originally proposed (Routes A and B; Scheme 6).<sup>16</sup> The results were consistent with the possibility of a third "two-chain" route from triketide and pentaketide intermediates (Route C) or from a linear octaketide folded as shown in route D.



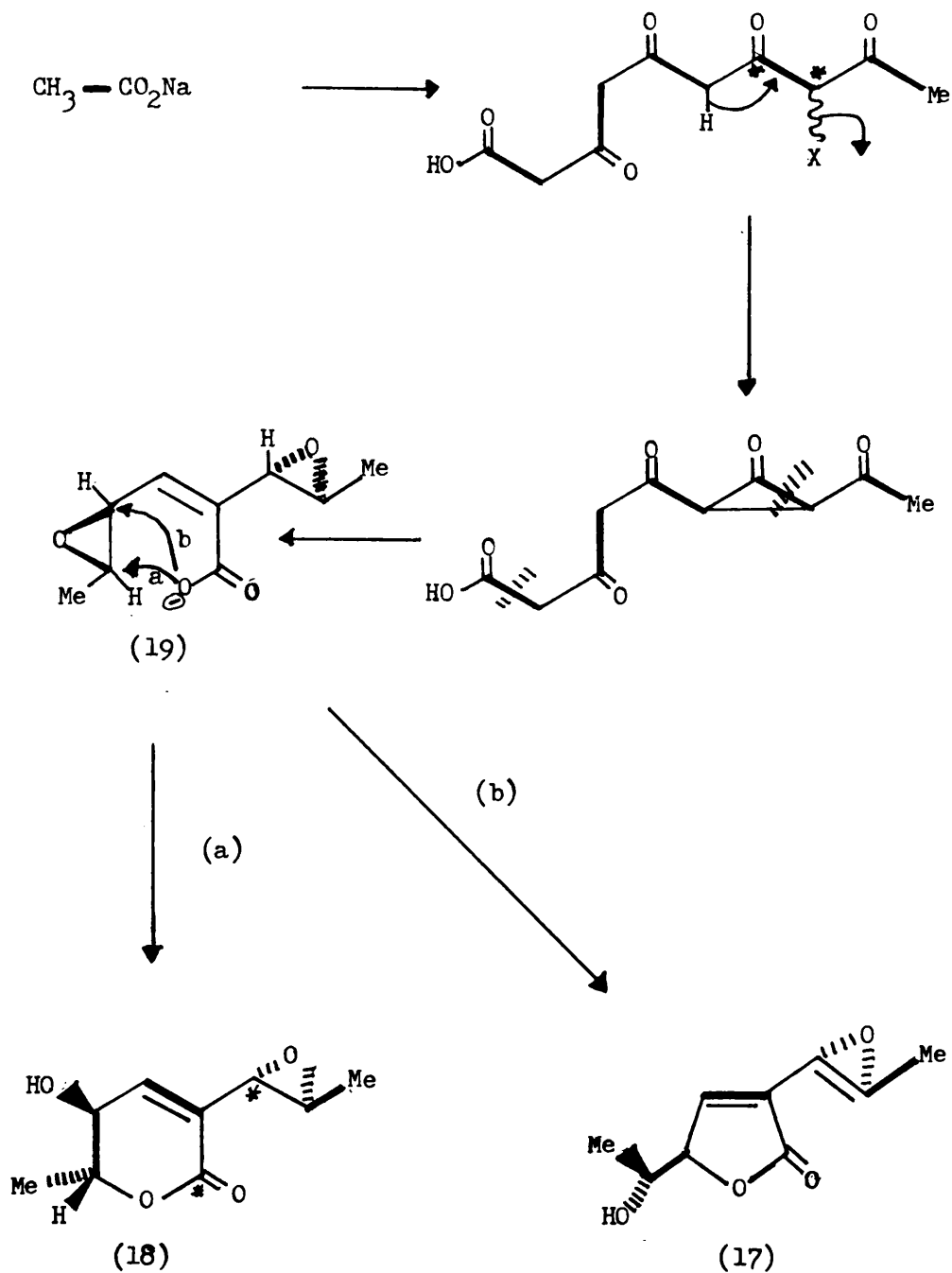
A further example of the use of  $^{13}\text{C}$ -labelled acetate was in the investigation into the biosynthesis of aspyrone (17). Aspyrone has been isolated from the ochratoxin-producing strain of Aspergillus ochraceus.

Incorporation of  $[1-^{13}\text{C}]$ -,  $[2-^{13}\text{C}]$ -, and  $[1,2-^{13}\text{C}_2]$ -acetate into aspyrone by Aspergillus melleus suggests its formation from three intact acetate units and three carbons derived from cleaved acetate units.<sup>17</sup>

An unusual mechanism was originally suggested to account for this labelling pattern which involved the introduction and subsequent loss, of a  $\text{C}_1$ -unit into a pentaketide precursor with cyclisation, rearrangement and ring cleavage. The alternative pathway of scheme 7, a Favorskii rearrangement of a linear pentaketide and loss of the terminal carboxyl group, was subsequently proposed.<sup>18</sup>

Support for this route was gained from the detection in the n.m.r. of a long range (two bond)  $^{13}\text{C}$ - $^{13}\text{C}$  coupling between carbon atoms of the rearranged acetate unit (\*). The pattern of incorporation of  $[1,2-^{13}\text{C}_2]$  acetate into asperlactone (18), also a metabolite of Asp. melleus is similar to aspyrone.<sup>19</sup> Staunton suggested that the two compounds may have a common precursor of type (19) which can cyclise by either route (a) or (b) to aspyrone and asperlactone, respectively.





Scheme 7.

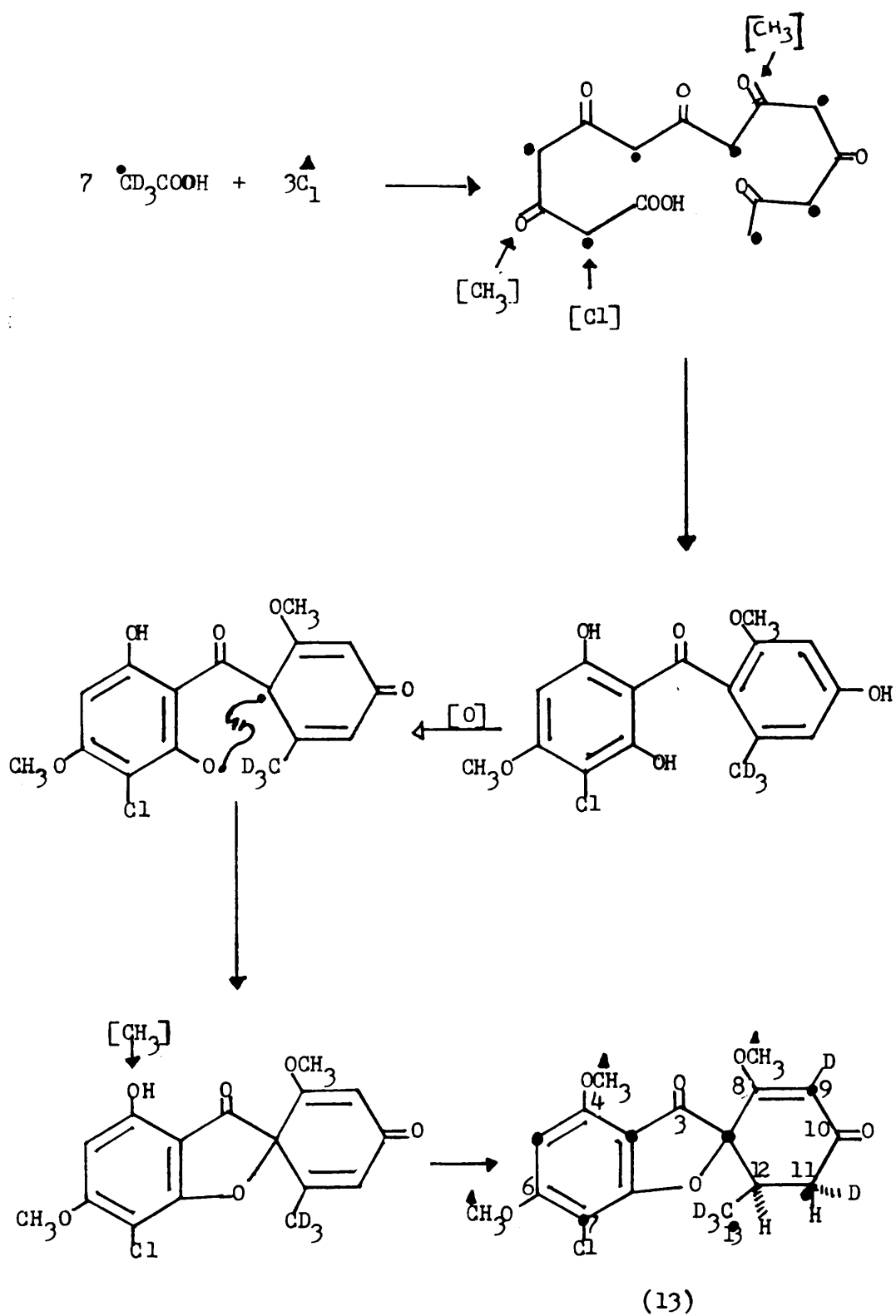
### Deuterium N.M.R.<sup>20</sup>

The potential of deuterium n.m.r. was first illustrated in 1964 but it is only in the last decade or so that it has been widely employed by organic chemists.<sup>21</sup> Deuterium, is a quadrupole nucleus with a nuclear spin of 1. It has the advantage of being an inexpensive, stable isotope and does not require any special handling techniques. It has a low natural abundance, 0.016 per cent, enabling the incorporation of deuterium precursors to be positively identified. The incorporation of molecules singly labelled with  $^{13}\text{C}$  can only be detected with confidence at dilution levels of less than one-hundred fold. Thus  $^2\text{H}$  n.m.r. is effectively sixty times more sensitive than  $^{13}\text{C}$  n.m.r. when applied to biosynthetic study. Deuterium also exhibits short relaxation times and no nuclear Overhauser effect allowing it to be accurately integrated. Consequently the extent of enrichment in a partially deuteriated molecule may be easily determined via integration.

There are however, two major limitations of deuterium n.m.r. The line width is much broader than that of  $^1\text{H}$  thus leading to poor resolution in the spectrum. There is also much more spectral crowding than in  $^1\text{H}$  since the sensitivity is low ( $1/100$  of  $^1\text{H}$ ) and the chemical shift scale and coupling constants are only one-sixth of the value of  $^1\text{H}$ .

The biosynthesis of the fungal metabolite

griseofulvin (13) produced by Penicillium urticae has been extensively studied using deuteriated precursors. Griseofulvin produced, when  $[2-^2\text{H}_3]$  acetate is fed to P. urticae is expected to be deuteriated at C-5, C-9, C-11 and C-13. The  $^2\text{H}$  n.m.r. spectrum shows that these positions are indeed labelled. Some washout was noted but the label is also incorporated to some extent in the methoxy groups at C-4, C-6, and C-8. It was also found that C-5 was exclusively labelled in the  $\alpha$ -position demonstrating that reduction of the intermediate dehydrogriseofulvin takes place exclusively in the trans fashion (Scheme 8).



Scheme 8.

### Carbon 13 - Deuterium N.M.R. Approach.

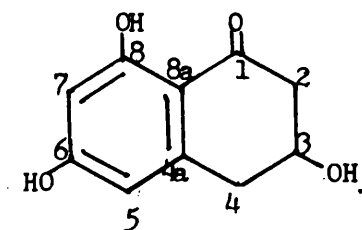
An attractive alternative to the techniques discussed previously is to monitor the presence of isotopic hydrogen (mainly deuterium) indirectly through its interaction with the adjacent carbon nucleus. A precursor is chosen so that the isotopically labelled site is enriched with  $^{13}\text{C}$ ; the presence of the hydrogen isotope in the biosynthetic product is thus detected by its coupling to  $^{13}\text{C}$  in the  $^{13}\text{C}$  n.m.r. spectrum. This approach has mainly been applied to deuterium and has an important advantage in that the labelling pattern of individual molecules can be deduced from the multiplicity and chemical shift values of the n.m.r. signals.

In a proton decoupled  $^{13}\text{C}$  n.m.r. spectrum, a carbon with one directly attached deuterium appears as a triplet whose lines are of equal intensity, this is because the nuclear spin of deuterium is one.<sup>22</sup> The  $J(^{13}\text{C}-^2\text{H})$  values are one-sixth of the equivalent  $J(^{13}\text{C}-^1\text{H})$  values and the signal is centred 0.3-0.6ppm upfield; the multiplicity is also increased. Thus  $\text{C}^{\text{D}}_2$  will be seen as a quintet and  $\text{C}^{\text{D}}_3$  as a septet. A partially deuteriated methyl will give rise to a complex signal comprising lines from  $-\text{C}^{\text{D}}_3$ ,  $-\text{C}^{\text{D}}_2\text{H}$ ,  $-\text{C}^{\text{D}}\text{H}_2$  as well as a  $-\text{CH}_3$  labelled species. However running the spectrum with deuterium decoupling instead of proton decoupling can simplify spectral analysis a great deal.

$^{13}\text{C}$ - $^2\text{H}$  N.m.r. is limited by the relative weakness of the  $^{13}\text{C}$ - $^2\text{H}$  signal in the n.m.r. spectrum. This is mainly due to three factors:-

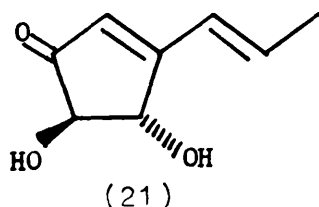
- (i)  $^{13}\text{C}$ - $^2\text{H}$  relaxation is much less efficient than  $^{13}\text{C}$ - $^1\text{H}$  relaxation.
- (ii) The replacement of hydrogen by deuterium is accompanied by loss of N.O.E. enhancement.
- (iii) Increased multiplicity causes loss of signal strength in individual lines.

This technique has been employed quite widely in biosynthetic studies, for example in the incorporation of  $[2\text{-}^{13}\text{C}, 2\text{-}^2\text{H}_3]$  acetate into the fungal metabolite skylactone (20). The enriched compound showed signal enhancements for C-2, C-4, C-5, C-7 and C-8a, but the signal intensities for C-4 and C-5 were lower than expected, indicating the presence of deuterium. A triplet was observed 0.3 ppm upfield from the normal  $^{13}\text{C}$  signal for C-4 but no corresponding deuteriated signal was visible for C-5. Rerunning the deuterium decoupled spectrum showed the signal at C-4 to be a doublet indicating a  $-\text{CHD}-$  while a singlet at 108.7 ppm was able to be assigned to molecules carrying a deuterium at C-5.



### Ring Cleavage Products.

As already mentioned there are an extremely large number of polyketide-derived natural products which are or contain an aromatic ring in their structure. However, there are in fact many interesting examples which do not, among which are those compounds containing five-membered rings. Quite a few of these cyclopentane-type compounds are formed by the extrusion of a carbon atom from a six-membered aromatic precursor. This type of ring cleavage reaction is extremely interesting to the chemist and has been the subject of many biosynthetic investigations. There are numerous examples of five-membered ring containing natural products in the literature which are of polyketide origin. One of these which is of particular interest to us is the natural product terrein (21) whose biosynthesis is the subject of this dissertation. It is thought that terrein is derived by way of a ring contraction of a six-membered aromatic-type precursor. In the forthcoming pages a brief survey will look at other natural products from the literature which have been shown to be or contain a cyclopentane ring which has been formed by way of a ring cleavage type reaction.

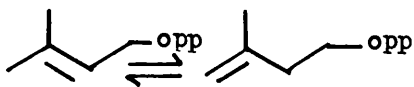
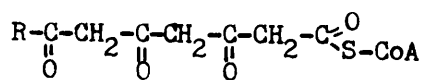
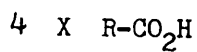


### Humulones.

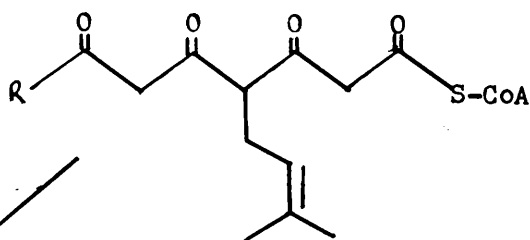
The hop plant, Humulus lupulus has for centuries been employed to give flavour to fermenting liquors. It is best known as the principal source of the aroma and bitter flavour of beer. The main constituents of the hop resin are the humulones ( $\alpha$ -acids) (22) and the lupulones ( $\beta$ -acids) (23). Drawert<sup>24,25</sup> in 1976 investigated the biosynthesis of these hop bitter components and proposed that the formation of the six-membered ring proceeds via a polyketide type pathway. He suggested that leucine is the precursor of the isovaleryl residue in the hop bitter compounds; the same being true for valine and isoleucine as precursors for the isobutyryl and 2-methyl butyryl residues.<sup>26</sup> He proposed that the humulone (22) could be biosynthesised from acetate via the pathway shown in scheme 9.<sup>27</sup>

It is thought that the  $\beta$ -acids actually contribute little to the bitterness of the beer, this is mainly due to the  $\alpha$ -acids.<sup>28</sup> The  $\alpha$ -acids may be transformed via alkaline hydrolysis into the humulinic acid (25). It is the isohumulones (23) which are thought to be responsible for at least seventy per cent of the bitterness of beer, when hops are boiled with brewers wort, the  $\alpha$ -acids become transformed into a resinous material now characterised as isohumulone. The compounds have been isolated as intermediates on the route to the production of humulinic acid via alkaline hydrolysis of humulone. During the storage of hops, the resins, in particular

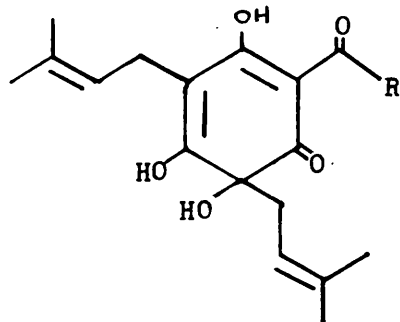
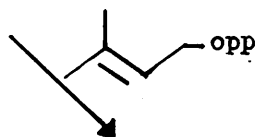
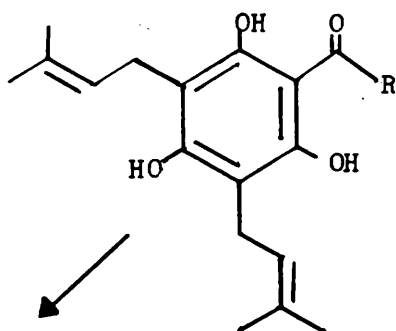
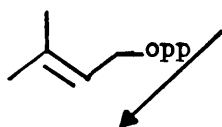
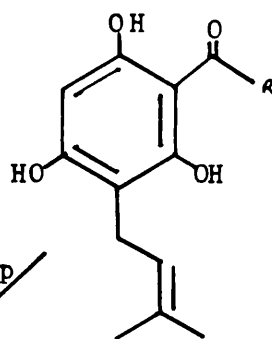
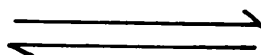
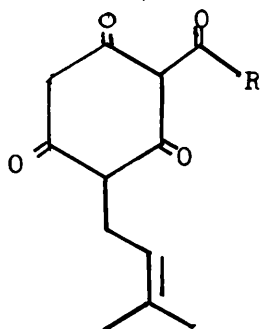




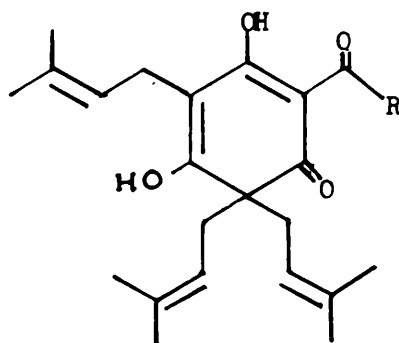
PP<sub>i</sub>



-CoASH



HUMULONE (22)

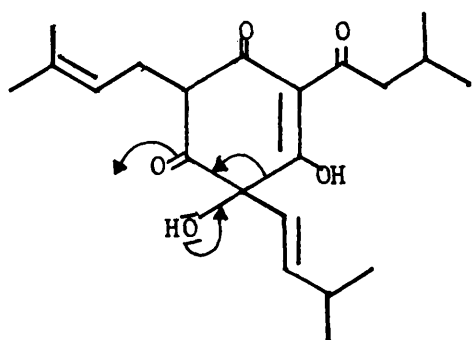


LUPULONE (23)

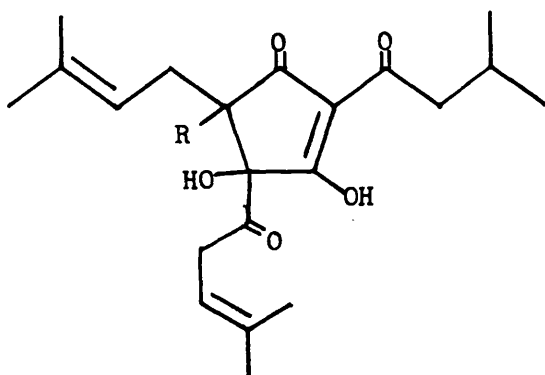
Scheme 9

the  $\alpha$ -acids undergo oxidation to yield compounds such as humulinone (24). The oxidation product of lupulones being hulupones (26).

Of particular relevance to this discussion is the fact that the six-membered ring humulones are easily rearranged to the five membered cyclopentanone derivatives (23), (24), (25) and (26) via a ring cleavage reaction.

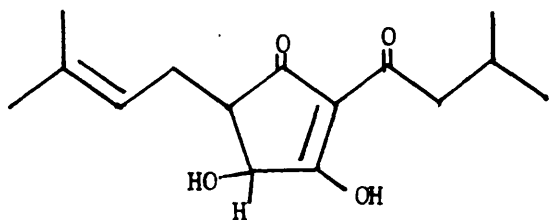


humulone (22)

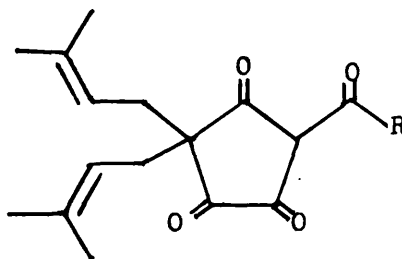


R=H isohumulone (23)

R=OH humulinone (24)



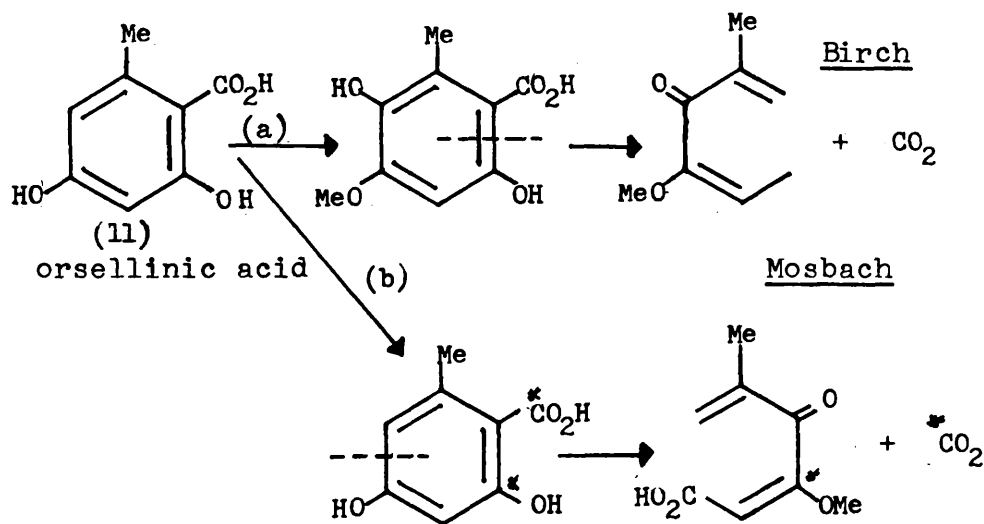
humulinic acid (25)



hulupone (26)

## Penicillic Acid

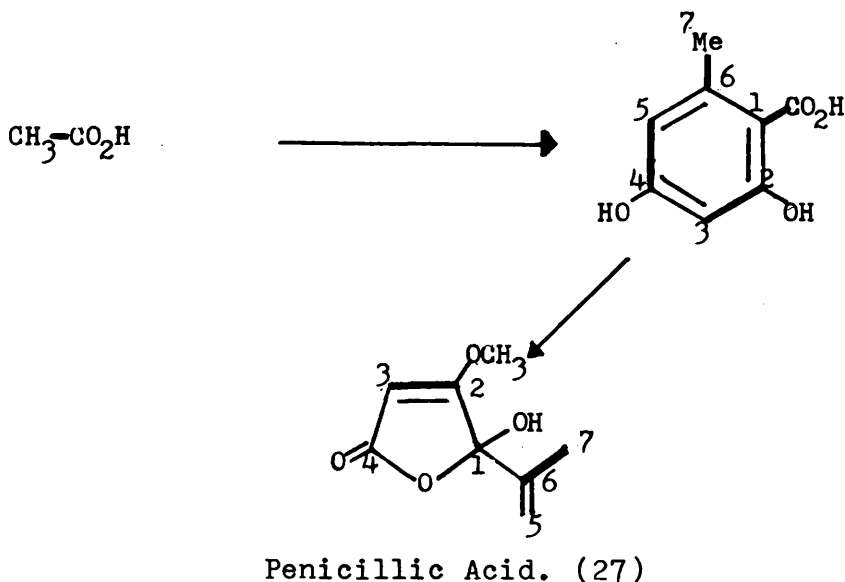
Penicillic acid (27) is an antibiotic and potent carcinogen produced by a wide variety of fungi.<sup>29</sup> It was first isolated in 1913 from Penicillium puberulum and its structure established by Raistrick<sup>30</sup> who obtained it from Penicillium cyclopium. It was not immediately apparent that penicillic acid was of polyketide origin but in 1958 Birch<sup>31</sup> established its acetate origin and suggested that it might be derived from orsellinic acid (11). Mosbach in<sup>32</sup> 1960 confirmed that orsellinic acid is indeed converted into penicillic acid by Penicillium barnese but found that cleavage of the aromatic ring was as shown in scheme 10 (Route b) and not as suggested by Birch (Route a).



Scheme 10.

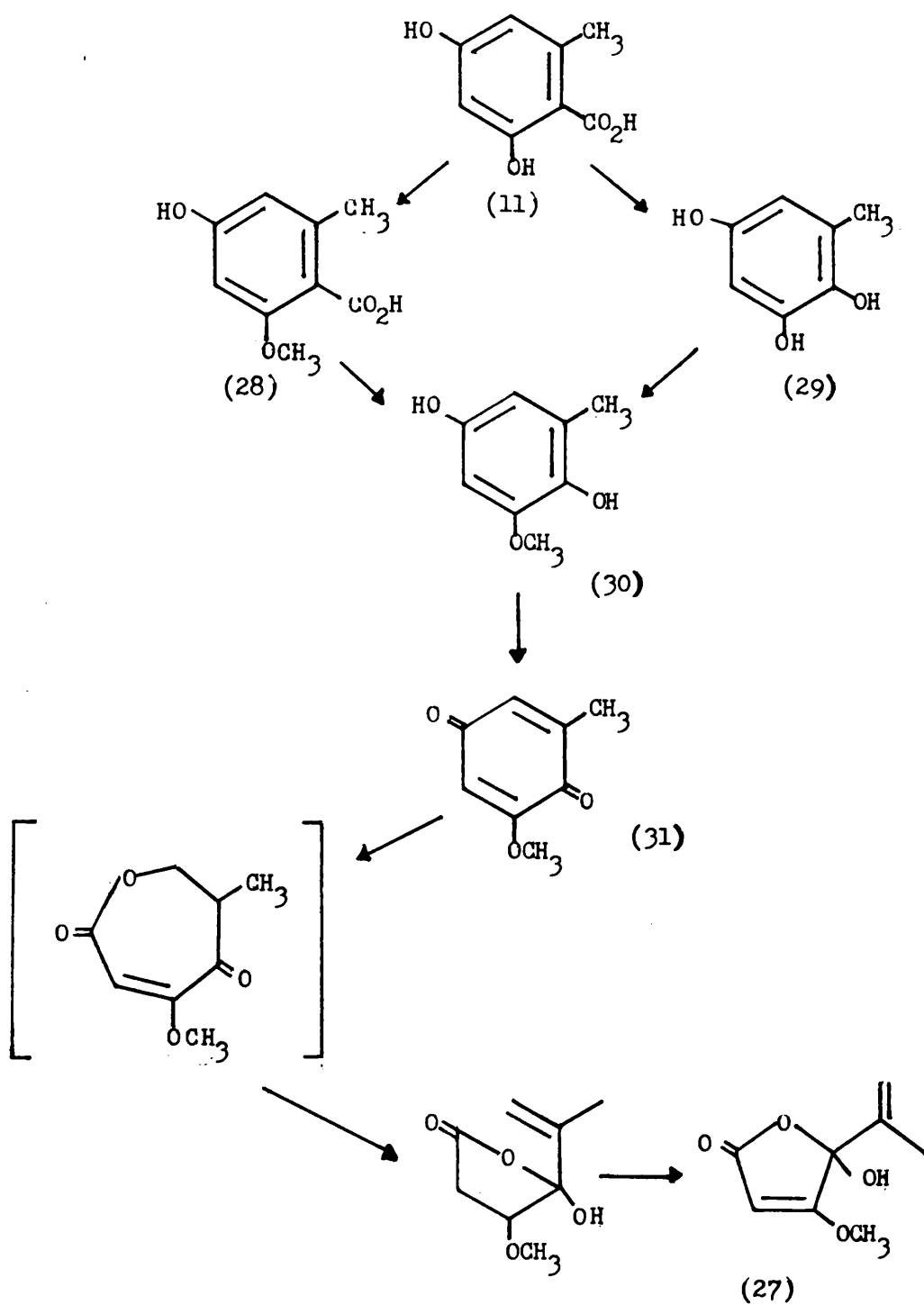
The number of intact acetate units in penicillic acid was determined by feeding [1,2-<sup>13</sup>C<sub>2</sub>] acetate.<sup>33</sup> Coupling in the <sup>13</sup>C n.m.r. was seen between C-2 and C-3 and also between C-6 and C-7, thus indicating two intact

acetate units were present in penicillic acid.



There has been much speculation over the years as to the correct order of the steps involved in penicillic acid biosynthesis. Gatenbeck<sup>34,35</sup> proposed that orsellinic acid (11) was methylated at the 2-hydroxy position followed by decarboxylation coupled with a hydroxylation reaction. The hydroquinone (30) could then be further oxidised to the quinone (31) which would then undergo ring cleavage to give penicillic acid (27). However recently Sekiguchi<sup>36</sup> found that <sup>14</sup>C-labelled 6-methyl-1,2,4-benzenetriol (29) was incorporated into Penicillium cyclopium producing penicillic acid. The metabolite (29), its methyl ether (30) and the benzoquinone (31) have all been incorporated into Penicillium cyclopium. However, 1-methylorcinol (28) was found not to be incorporated. Thus a new pathway was proposed for the production of penicillic acid (Scheme 11).

Biosynthesis of Penicillic Acid

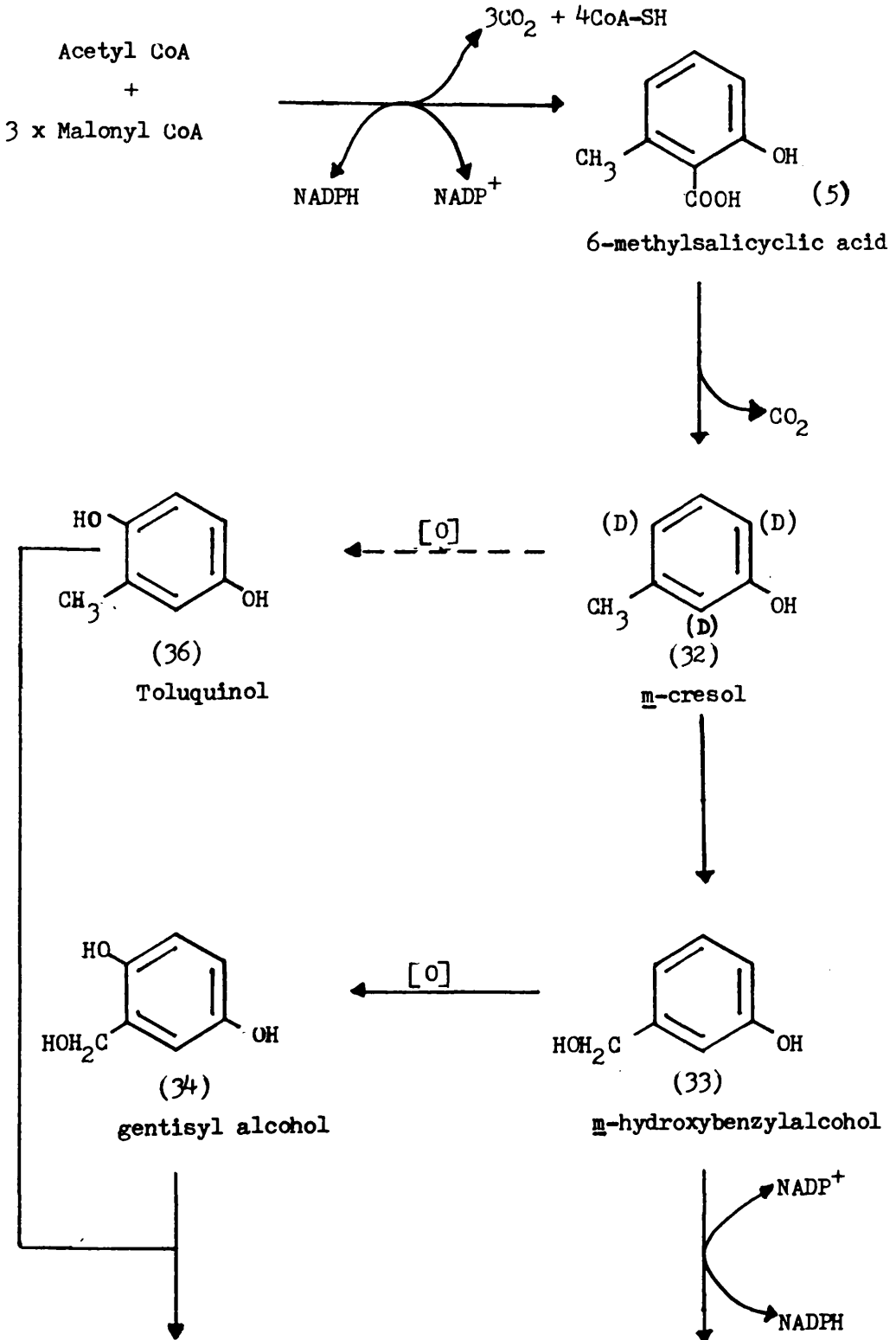


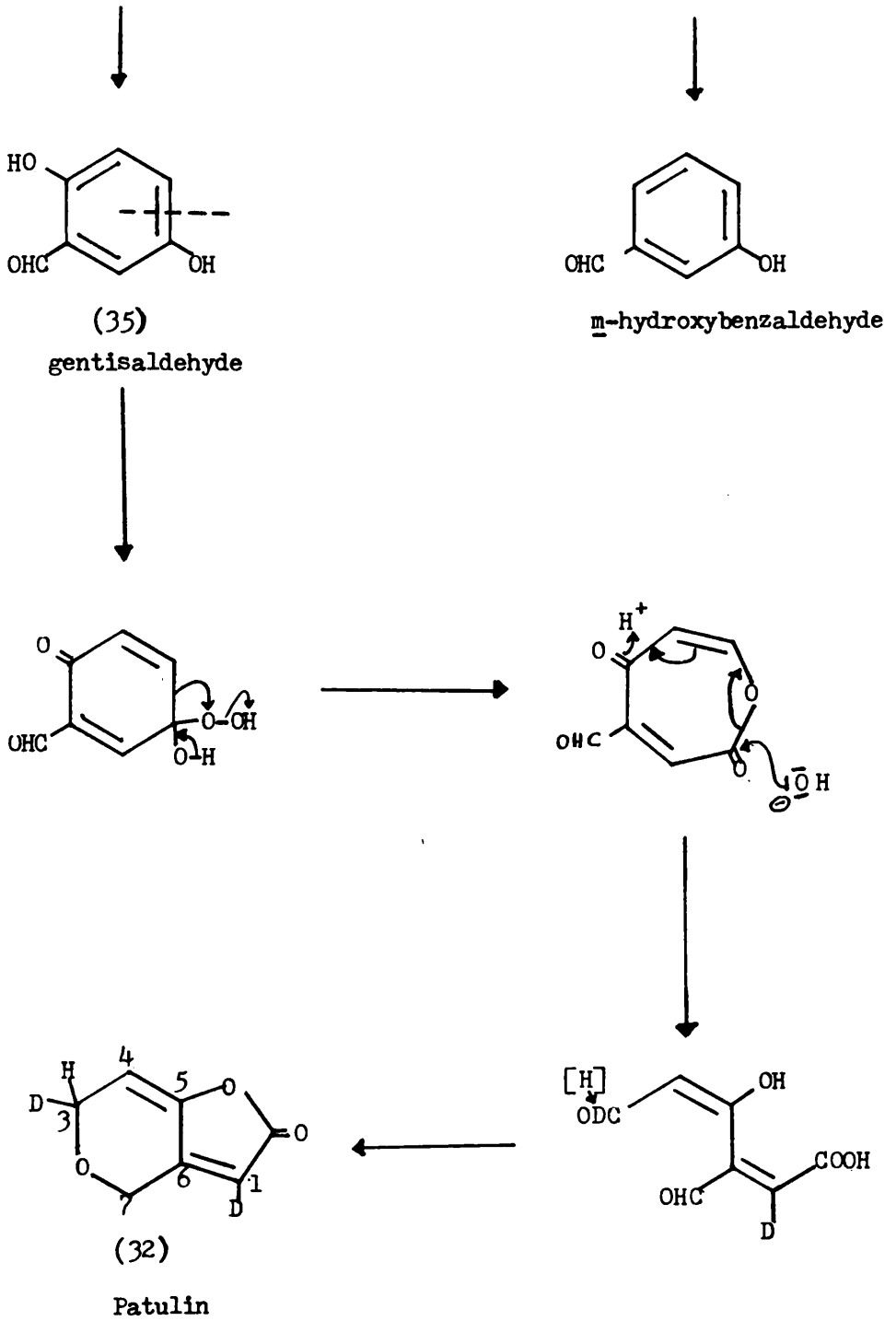
Scheme 11

### Patulin.

Patulin (32) has been isolated from many members of the fungi imperfecti as a result of its pronounced antibacterial activity in vivo. It is a potent carcinogen and occurs in mouldy apples. It is also stable in many apple-containing products such as pies and juices, and may be potentially very dangerous to human health. Patulin's structure was first elucidated by Woodward and Singh<sup>37</sup> in 1949. Its biosynthesis was then investigated by Birkenshaw<sup>38</sup> in 1953 who suggested that it may be derived by way of an aromatic precursor. Bu'lock and Ryan<sup>39</sup> confirmed this and showed that 6-methylsalicylic acid was converted into patulin (32) by Penicillium patulum. Scott and Yalpani<sup>40</sup> used deuterium labelled precursors followed by mass spectroscopic analysis of the products to follow the biosynthesis of patulin. They found that m-cresol (32), m-hydroxybenzylalcohol (33), gentisylalcohol (34) and gentisaldehyde (35) were all cometabolites of Penicillium patulum, being incorporated well into patulin. The route shown in scheme 12 via toluquinol (36) appears to be due to a side reaction taking place. When  $[2,4,6-^2\text{H}_3]\text{-}\underline{\text{m}}$ -cresol was fed to a glucose deficient medium it was found that up to 57% incorporation was observed. The fragmentation pattern of patulin is very well understood and it was therefore possible to locate the position of labelled isotopes quite easily in the molecule. The pathway shown in scheme 12 was

Biosynthesis of Patulin





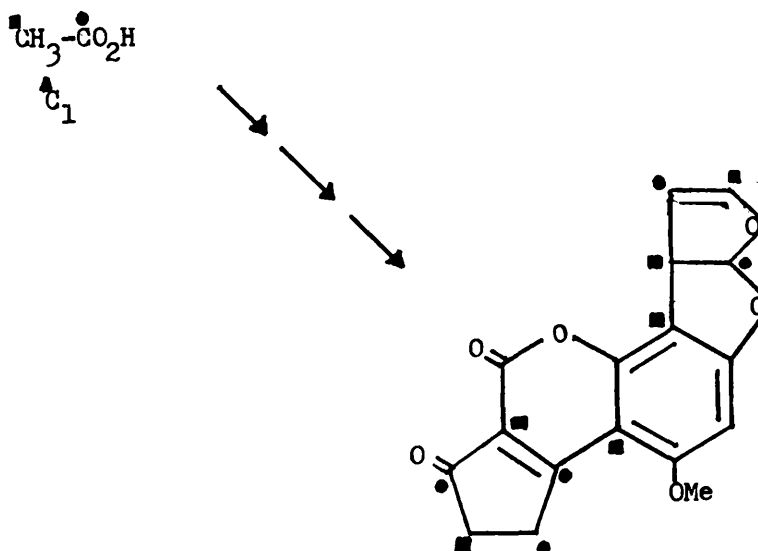
Scheme 12.



proposed for the biosynthesis of patulin.

### Aflatoxins

Aflatoxins are a group of fungal metabolites with homogeneous structural, biogenetic and toxological properties. They are produced by the imperfecti fungi of the Aspergillus type, A. versicolor and A. flavus. Aflatoxins are highly toxic to animals which may ingest them on eating mouldy food. They are now thought to be one of the most carcinogenic agents known to man, being active in rats at a level of 1  $\mu\text{g}$  per day. The biosynthesis of aflatoxins has been studied by Buchi<sup>35,36</sup> who as a result of elegant degradative experiments was able to establish the origin of the majority of carbon atoms in aflatoxin B (37).

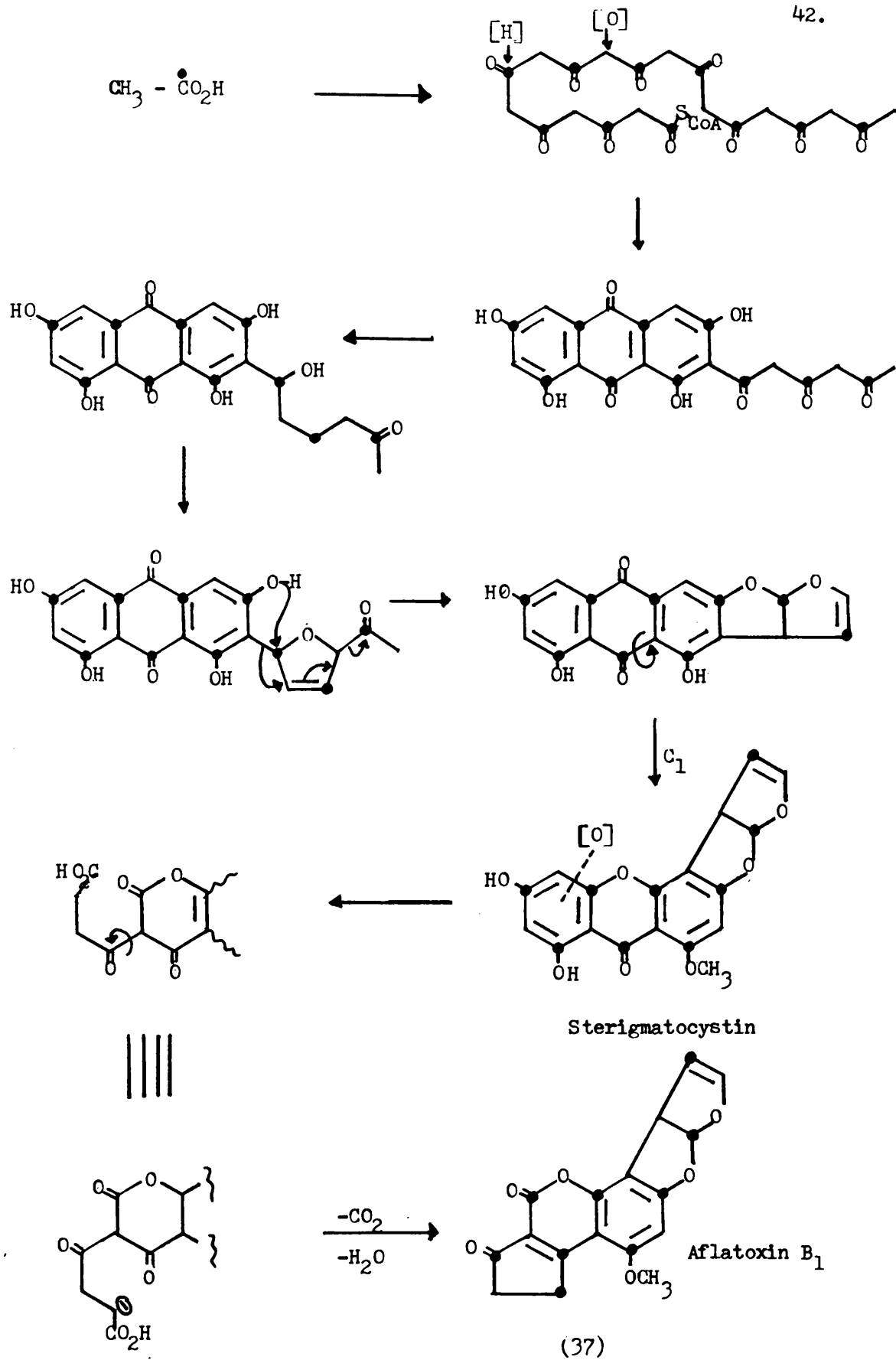


(37)

Of most interest to us is the fact that aflatoxins possess a five membered cyclopentenone ring system. It is thought that this ring moiety is derived via oxidative ring cleavage of the aromatic ring in sterigmatocystin. Sterigmatocystin itself is known to be converted into aflatoxin B, by cultures of Aspergillus parasiticus. A possible route to sterigmatocystin and consequently aflatoxins was proposed after  $^{13}\text{C}$ - and  $^{14}\text{C}$ - studies by Buchi, Holker and Tanabe.<sup>40</sup> [1,2- $^{13}\text{C}$ ] Labelled acetate was used to identify the number of intact acetate units. They proposed the biosynthetic pathway shown in scheme 13 for aflatoxin biosynthesis.

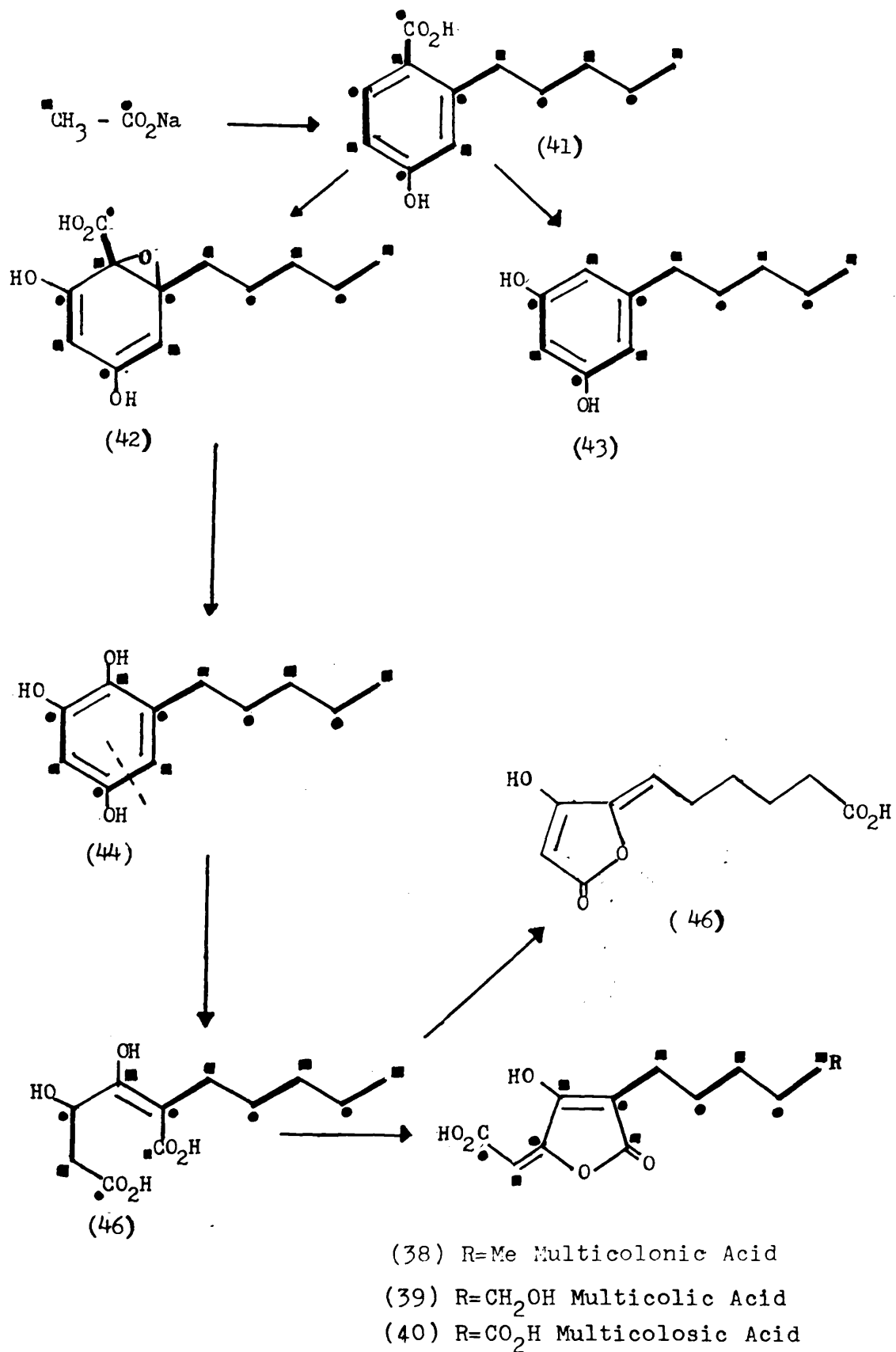
#### Tetronic Acids.<sup>41</sup>

Multicollic, multicolosic and multicolonic acids are metabolites of the fungus Penicillium multicolor. Their structures have been confirmed by Holker via  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. to be (38) (39) and (40) respectively. Holker also investigated the possibility that these three metabolites were biosynthesised from acetate, via oxidative fission of a pre-formed aromatic precursor, 6-pentylresorcylic acid (41). [ $1\text{-}^{13}\text{C}$ ] Acetate, [ $2\text{-}^{13}\text{C}$ ] acetate and [1,2- $^{13}\text{C}$ ] acetate were fed to Penicillium multicolor and the metabolites isolated, spectroscopically analysed. The  $^{13}\text{C}$  n.m.r. spectrum from the [ $1\text{-}^{13}\text{C}$ ]- and [ $2\text{-}^{13}\text{C}$ ] acetate derived samples showed that all carbons of the multicollic and multicolosic



Scheme 13.

acid skeletons were acetate-derived. From the spectra of the  $[1,2-^{13}\text{C}]$  acetate derived samples, it could be seen from  $^{13}\text{C}$ - $^{13}\text{C}$  couplings that there were only four intact acetate residues. The complete absence of couplings for carbons 1, 3 and 11 indicate their origin from acetate units which have been cleaved during biosynthesis and rules out the possibility of 1,2-cleavage in (41). This precludes the biosynthetic intermediacy of any symmetrical aromatic intermediate such as 5-pentylresorcinol (43). These results led Holker<sup>42</sup> to the assumption that the poly- $\beta$ -ketide derived molecule, 6-pentylresorcylic acid (41) was a biosynthetic intermediate on the pathway to these acids. However it was still necessary to postulate that this intermediate was not at any time during the biosynthesis converted into the symmetrical intermediate 5-pentylresorcinol (43), since this would give scrambling of the  $^{13}\text{C}$ - $^{13}\text{C}$  couplings in the compounds derived from  $[1,2-^{13}\text{C}]$ -acetate. A possible intermediate was thought to be the trihydric phenol (44), which could arise from (41) via an arene oxide of the type (42). Ring scission would have to occur at the 4,5-position indicated in scheme 14 to give the diacid (45). Enol lactonisation could then lead either to the tetrone acids (38), (39) and (40) or a compound of type (46).

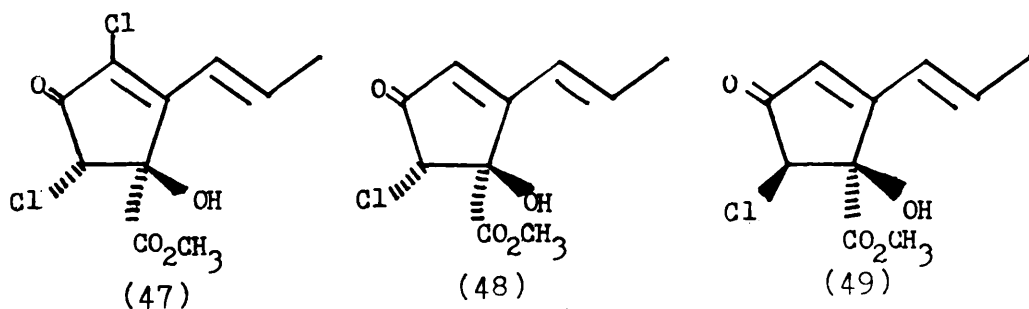


Scheme 14.

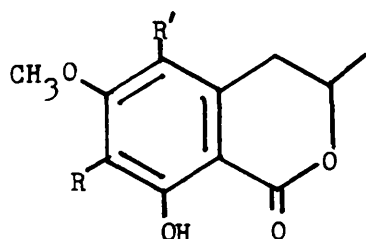
### Cryptosporiopsin

Several chlorinated metabolites containing five-membered rings have been isolated from cultures of Sporormia affinis and Periconia macrospinos.<sup>43</sup> Many of these metabolites have very similar ring structures to the five-membered ring compound terrein, which is the subject of this dissertation.

The most abundant metabolite isolated from S. affinis was the five-membered cyclopentenone derivative, cryptosporiopsin (47). Found also in smaller amounts were the two cyclopentenones (48) and (49).



As well as the five membered ring compounds shown above, three isocoumarins were also isolated from cultures of S. affinis. Two were found to be chlorinated, (50) and (51), the other being the dihydro-isocoumarin (52). It is thus tempting to suggest that a biogenetic sequence may link these two types of metabolite together.

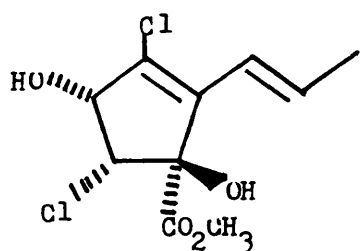


(50)  $R=R'=Cl$

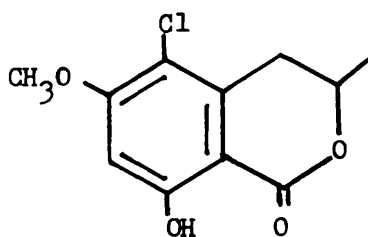
(51)  $R=Cl$  ;  $R'=H$

(52)  $R=R'=H$

At approximately around the same time, work with the fungus Periconia macrospinosa yielded two major metabolites.<sup>44</sup> The alcohol (53) which is now known as cryptosporiopsinol was found to be one of the metabolites, the other being the chlorinated isocoumarin (54). It was found that oxidation of compound (53) led to the ketone cryptosporiopsin which is already a metabolite of S. affinis, cryptosporiopsin itself has also been found to be present in crude extracts of P. macrospinosa.



(53)



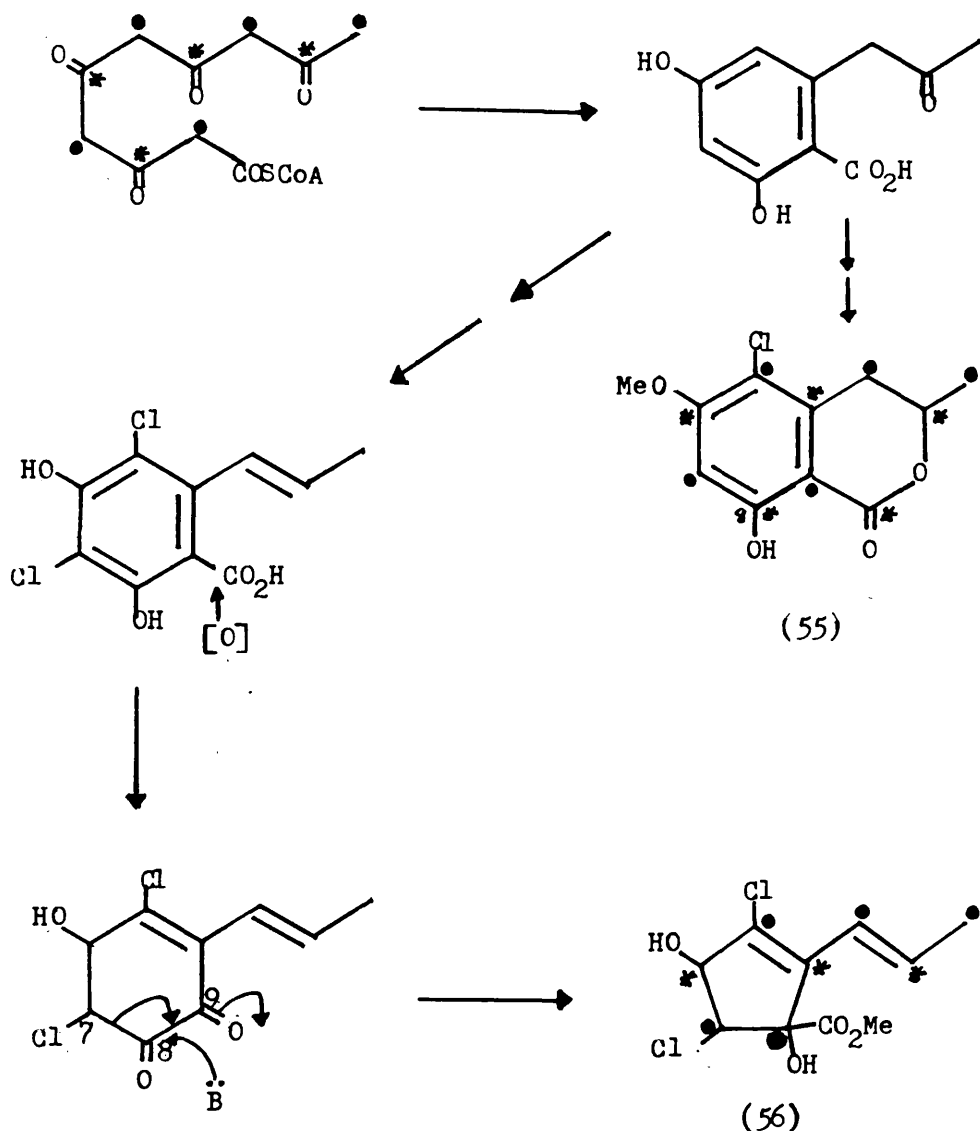
(54)

In 1975 Holker and Young<sup>45</sup> studied the biosynthesis of metabolites produced by Periconia macrospinosa. Previous to this it had already been suggested that cryptosporiopsin (47) was biosynthesised by contraction of an aromatic ring via a precursor which is structurally related to the co-occurring dihydroisocoumarin. Holker used cultures of P. macrospinosa which had been supplemented with  $[1-^{13}\text{C}]$ -,  $[2-^{13}\text{C}]$ - and  $[1,2-^{13}\text{C}]$ -acetate. The metabolites (55) and (56) were isolated and found to be enriched with approximately 1.3 and 1.6% respectively, excess  $^{13}\text{C}$  abundance at each labelled position.

Structural analysis of the dihydroisocoumarin (55) suggests its penta- $\beta$ -ketide origin since the  $[1,2-^{13}\text{C}]$ -acetate enriched sample showed five pairs of intact acetate units.  $[1-^{13}\text{C}]$ -acetate and  $[2-^{13}\text{C}]$ -acetate showed an alternative labelling pattern in the isocoumarin consistent with polyketide biosynthesis.

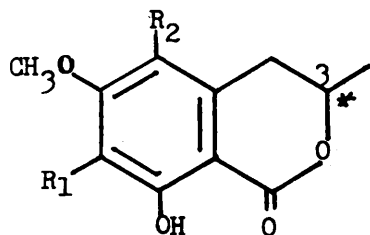
However of greater interest was the fact that cryptosporiopsin (56), derived from  $[1,2-^{13}\text{C}]$ -acetate showed only three intact acetate units. Feedings with  $[1-^{13}\text{C}]$ - and  $[2-^{13}\text{C}]$ -labelled acetate showed a break in the alternative labelling pattern at C-7 and C-8 of cryptosporiopsinol as seen in scheme 15. This indicates that both carbons 7 and 8 must be derived from the methyl group of acetate. A plausible suggestion may be that a ring contraction of an aromatic precursor is occurring involving fission of the 7-8 bond, with extrusion of C-8 from the isocoumarin (55).





Scheme 15.

The intermediacy of the dihydroisocoumarin in the biosynthesis of cryptosporiopsinol<sup>(56)</sup> was investigated by Henderson.<sup>47</sup> He synthesised the four naturally occurring dihydroisocoumarins (57) - (60) with a  $^{14}\text{C}$ -label at C-3 and then monitored their incorporation by Periconia macrospinosa. It was also of interest to establish the relevance of the methoxy group at C-6, so he also synthesised the O-demethylated derivatives (61) - (64) again with a  $^{14}\text{C}$ -label at C-3.

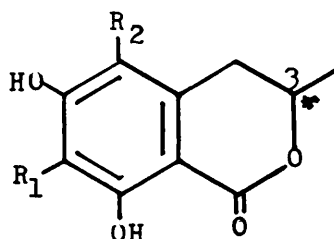


(57)  $R_1=R_2=H$

(58)  $R_1=Cl$  ;  $R_2=H$

(59)  $R_1=H$  ;  $R_2=Cl$

(60)  $R_1=R_2=Cl$



(61)  $R_1=R_2=H$

(62)  $R_1=Cl$  ;  $R_2=H$

(63)  $R_1=H$  ;  $R_2=Cl$

(64)  $R_1=R_2=Cl$

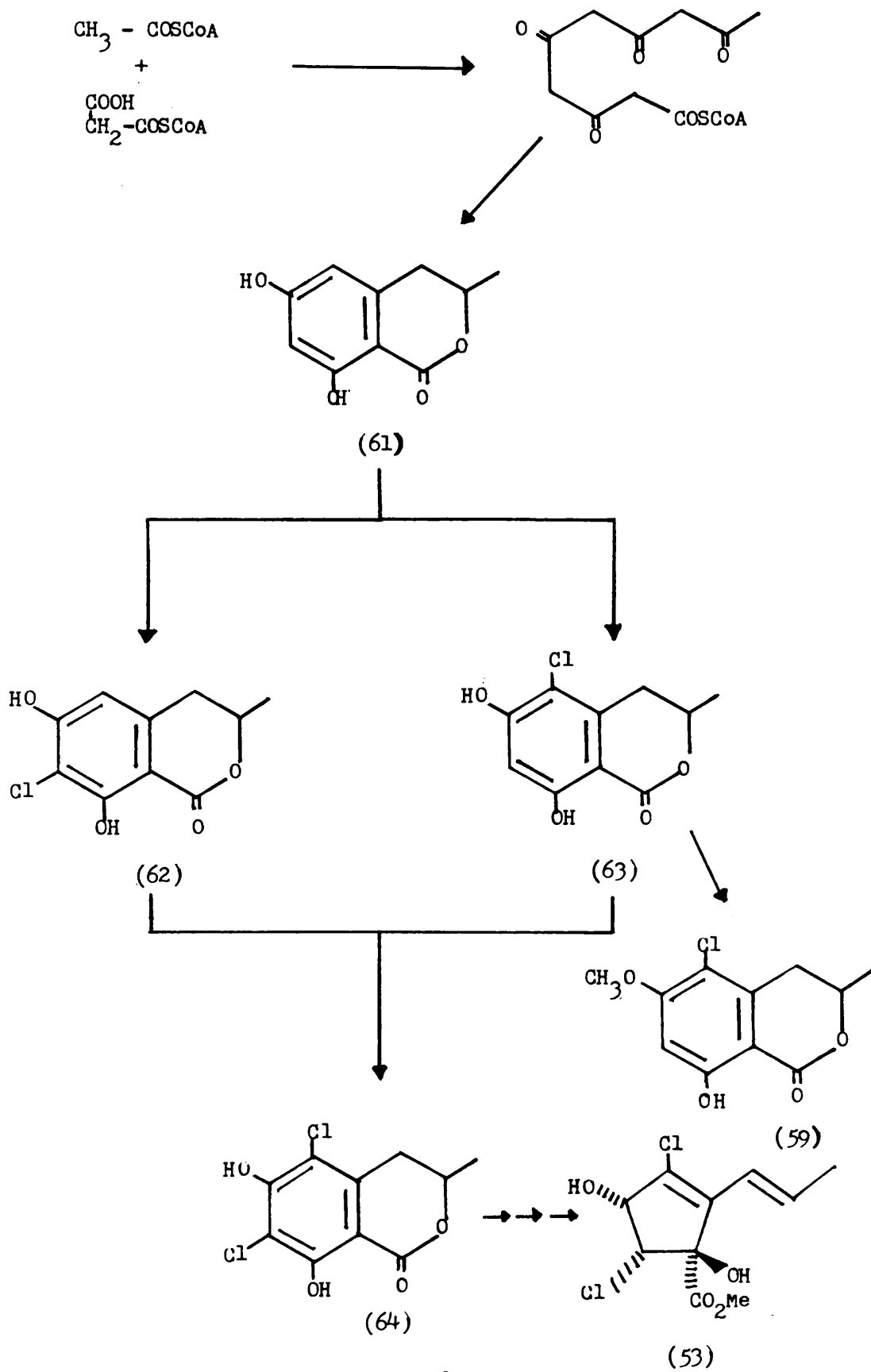
Of the eight labelled dihydroisocoumarins fed, only three (57), (61) and (63) were incorporated into the dihydroisocoumarin (55) at any significant level. However all isocoumarins were incorporated into cryptosporiopsinol to at least some extent. (64) was found to be the most efficiently incorporated precursor although the two monochloroderivatives (62) and (63) are both incorporated to a significant extent. The dihydroisocoumarin (61) and its 5-chloro derivative (63) are found to be incorporated far more efficiently than the 6-0-methyl derivative (57). Thus suggesting that chlorination of the ring is more probable after aromatisation but before O-methylation.

The isocoumarins (62) and (64) were tested for true intermediacy by precursor trapping experiments. This involves increasing the metabolic pool of a potential precursor then feeding radio-labelled acetate. The intermediate would then be trapped in a radio-labelled form if it is indeed acetate-derived and also if it is

a true intermediate. The metabolites (62) and (64) were found to be active on isolation thus demonstrating that they are indeed produced by P. macrospinosa and are both incorporated into cryptosporiopsinol. They are both likely to be true intermediates on the pathway to cryptosporiopsinol. Henderson's results allowed the following biosynthetic proposal to be put forward regarding the pathway to cryptosporiopsinol from acetate. (Scheme 16).

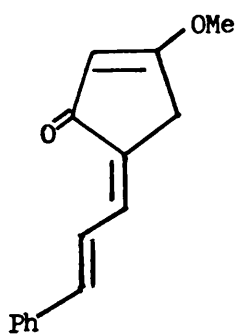
#### Less Common five-membered rings.

There are a number of other less common natural products present in nature which contain a five-membered ring system that may have been derived by way of a ring cleavage reaction. Examples of such compounds are those like lucidone (65) which is obtained from the extracts of the plant Lindera lucida<sup>48</sup>. Linderone (66) and its methyl derivative have been isolated from the roots of the Malaysian tree Lindera pipericarpa.<sup>49</sup> The seeds and bark of the tree have been used by natives for making cosmetic powders and the fruit as a flavouring agent as well as a substitute for cubebs in medicine. Bongkreikic acid (67) is another five-membered ring containing compound.<sup>50</sup> This is a toxic antibiotic produced by Pseudomonas cocovenaras on partially defatted coconut.

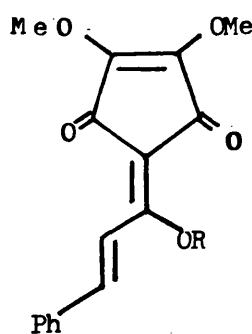


Scheme 16.

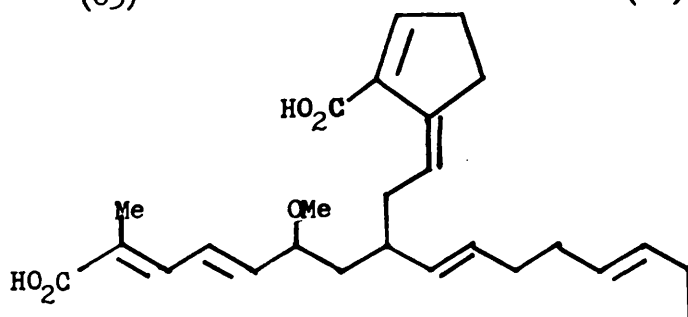
Finally, there is calythrone (68) which occurs in the steam-volatile oil of the myrtaceous Australian plant Calytrix tetragona.<sup>51</sup> Calythrone was first formulated by Birch<sup>52</sup> in 1951 as having the  $\beta$ -triketone structure (68). Calythrone is in fact the only exception to the occurrence of six-membered rings in  $\beta$ -triketones as demanded by the acetate hypothesis. The ready formation of a five-membered ring from a six-membered one has been already seen in the humulone series. By analogy with this and an additional dehydration stage involving a Wagner-Meerwein rearrangement, Penfold<sup>55</sup> put forward a plausible biogenetic route for the biosynthesis of calythrone seen in Scheme 17.



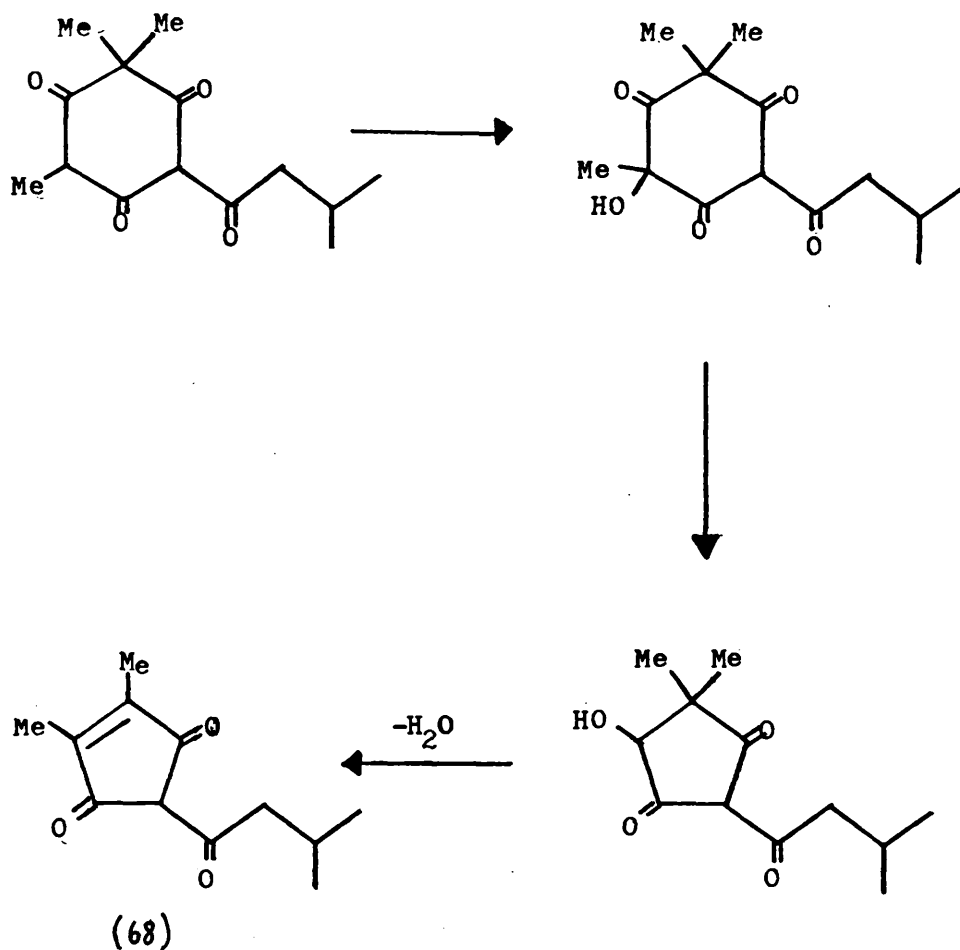
(65)



(66)



(67)



Scheme 17

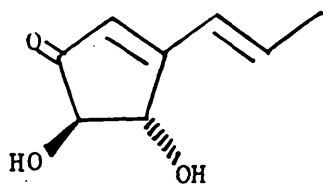
Since terrein is closely related in structure to many of the aforementioned cyclopentane derivatives especially those like cryptosporiopsinol (53) and its derivatives. It is therefore not unreasonable to imagine that the biosynthetic pathway to terrein may be somewhat similar to the pathways leading to some of the compounds mentioned previously. The remainder of this dissertation will concentrate on terrein and look at plausible biosynthetic pathways that may lead to this natural product. Attention will be given to the

synthesis of possible intermediates that may be involved in the biosynthesis of terrein and the nature of the ring contraction leading to terrein.

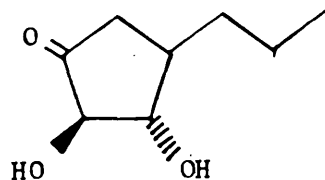
## Chapter 2.

### The Biosynthesis of Terrein.

The optically active cyclopentenone, terrein (21) is one of the major metabolites produced by the fungus Aspergillus terreus. Terrein was first isolated in 1935 from the same fungus by Raistrick and Smith<sup>54</sup> who also investigated its structure. In an extensive and elegant contribution Raistrick<sup>55</sup> and his collaborators established that tetrahydroterrein, which is easily obtained by catalytic hydrogenation of terrein, had the constitution (69). It wasn't however, until almost twenty years later that the correct structure was actually put forward for terrein. This was done simultaneously by two men, J.F. Grove<sup>56</sup> while working for I.C.I. in Hertfordshire and D.H.R. Barton<sup>57</sup> while at Birkbeck College in London. Both men suggested the same structure (21) for terrein but it was Barton who also elucidated the stereochemistry of terrein, both relative and absolute, by converting it into a derivative of (+)-tartaric acid.



(21)



(69)

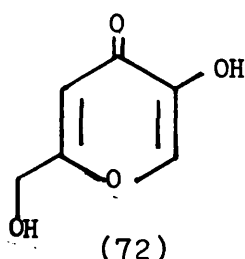
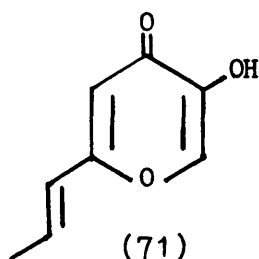
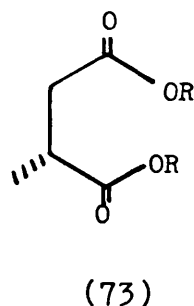
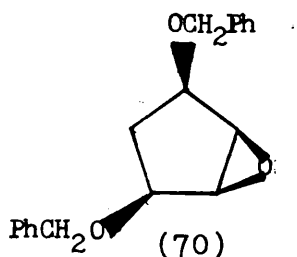
Terrein, itself does not exhibit any noticeable biological activity but is interesting as an enantio-



merically pure, highly functionalised cyclopentenoid building block, for example as prostaglandin precursors.

The synthesis of terrein proved to be problematical because of its high sensitivity towards acid and base.

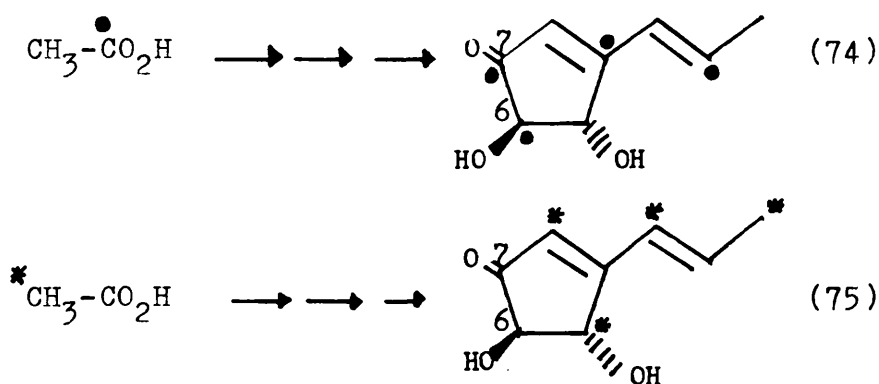
The first total synthesis of racemic terrein was achieved in 1974 by Weinreb.<sup>58</sup> However this was a rather laborious nine-step synthesis from cis-1,4-bisbenzyloxy-2,3-epoxycyclopentane (70) leading to (+) terrein in a low overall yield. Three years later Barton<sup>59</sup> came up with a different route to terrein involving photochemical ring cleavage of an appropriate 3-hydroxy-4-pyrone (71), which itself is readily synthesised from kojic acid (72). In 1981 Klunder<sup>60</sup> reported an efficient stereospecific total synthesis of (+) terrein employing cyclopentadienone epoxides as key intermediates. The most recent report of a synthesis of terrein was in 1990 by Altenbach.<sup>61</sup> He has synthesised optically active (+) terrein from the suitably protected L-tartaric acid derivative (73). It appears that his four step synthesis of optically active terrein is not only shorter and more effective than the other three previously known routes, but also that with intermediates readily accessible, enantiomerically pure five-membered ring building blocks are available in good yield. This could be useful for the synthesis of other target materials like carbocyclic nucleoside analogues.



The biosynthesis of terrein, has also presented a long standing and intriguing problem. It was Birch<sup>62</sup> in 1957 who first considered the formula of terrein and suggested that it arose at least partly by the polyketide-fatty acid mode of biosynthesis. Terrein is unusual in that it contains a five-membered ring, since polyketides usually contain six-membered rings, if any because they are connected by closures at the  $\beta$ -positions of the original chain.

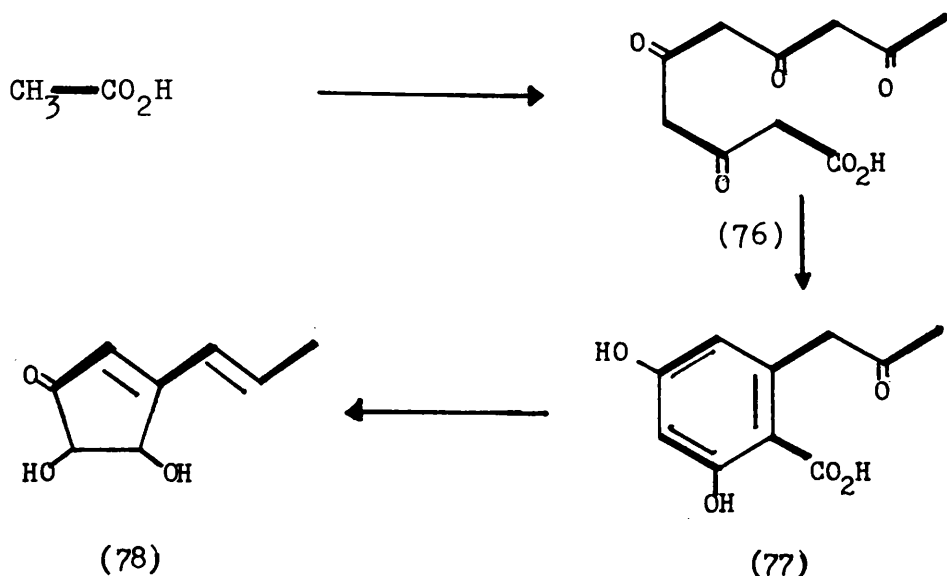
Birch proved his theory by feeding  $[1-^{14}\text{C}]$  acetate to Aspergillus terreus. Analysis of the distribution of the radiolabel showed that  $[1-^{14}\text{C}]$  acetate was incorporated well into terrein clearly showing that terrein was of polyketide origin. There was however, an unusual feature in the distribution of the  $^{14}\text{C}$  label, for the most part terrein was found to be labelled alternately as seen in Scheme 18 and the distribution of activity over the labelled positions was uniform.

However, there was a break in the alternate labelling pattern at C-6 and C-7 both of which contained a label indicating they must both be derived from the carboxyl group of acetate. Parallel incorporation studies with  $[2-^{14}\text{C}]$  acetate produced complimentary results and feeding with  $[2-^{14}\text{C}]$  malonate provided evidence for the starter unit being C-1-C-2 of the hypothetical ketide chain.



Scheme 18

Birch suggested that mechanistically this unusual distribution of the labels at C-6 and C-7 could probably be best explained by the contraction of a six-membered precursor to give the five-membered terrein. If it is assumed that a basic pentaketide intermediate such as (76) is involved, then a suitable scheme for terrein biosynthesis could be proposed involving (76) or perhaps a polyketide chain in which one of the ketone groups is reduced. This chain could then cyclise to give a possible six-membered aromatic precursor such as (77) which on extrusion of a carbon atom would lead to the formation of terrein (78) (Scheme 19).



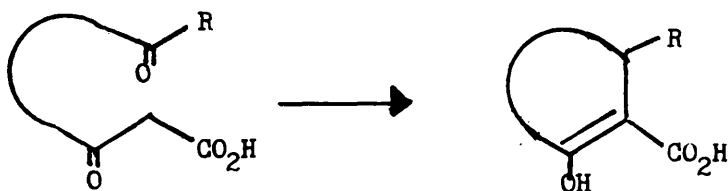
Scheme 19

Hill<sup>63</sup> and co-workers examined the incorporation of  $[1,2-^{13}\text{C}_2]$  acetate, an established technique for looking at structural change in polyketide biosynthesis. He obtained results which supported Birch's idea of a ring cleavage occurring in the formation of terrein. Interpretation of the  $^{13}\text{C}$  n.m.r. spectrum of terrein isolated from feedings with  $[1,2-^{13}\text{C}_2]$  acetate, showed that three  $\text{C}_2$ -acetate units were being incorporated intact, the remaining two carbons being derived from separate  $\text{C}_2$ -units.

If it is assumed that terrein is formed from a six-membered aromatic compound such as (77) seen in Scheme 19, then there must be at least four main steps taking place. These steps may occur in any order.

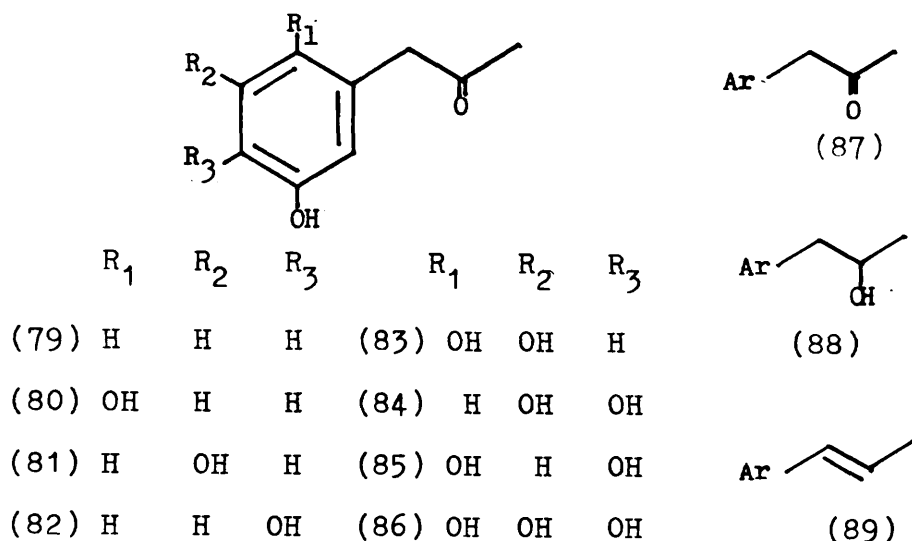
- i. Hydroxylation of the ring
- ii. Modification of the side chain
- iii. Decarboxylation
- iv. Ring Cleavage

In order to prove the theories suggested by Birch, Hill<sup>64</sup> synthesised a large number of aromatic precursors which could be possible intermediates on the pathway to terrein. Several possibilities were eliminated almost immediately. It is likely that hydroxylation of the ring will occur before the ring contraction step since enzymes which catalyse hydroxylation reactions in aromatic systems are often found to be present in fungi. On examination of all polyketides which arise by cyclisation involving a methylene group adjacent to the terminal carboxyl group of a polyketone chain, it was found that the oxygen atom next to the carboxyl group is always retained. Thus a hydroxy group must be present at this position in any proposed intermediate.

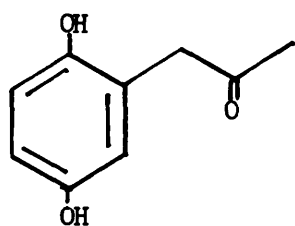


The remaining three positions of the aromatic ring could all possibly carry a hydroxyl group, thus allowing eight different hydroxylation patterns, though some are less likely than others. The nature of the side chain also needed some consideration; three possible groups need to be examined: the ketone (87), alcohol (88) and olefin (89). Decarboxylation is also

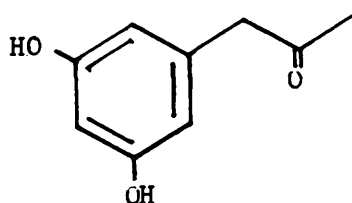
possible at any stage.



The first two compounds which were synthesised and fed were the aryl propanones (80) and (81). Results showed that these ketones were in fact degraded to acetate and then incorporated, thus they are not metabolites on the pathway to terrein.



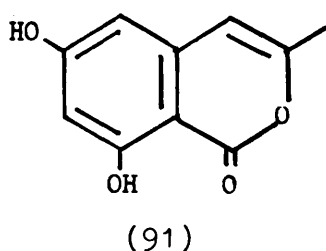
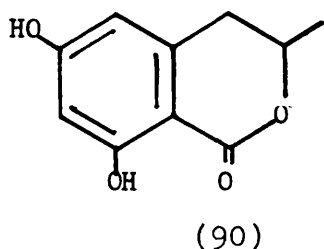
(80)



(81)

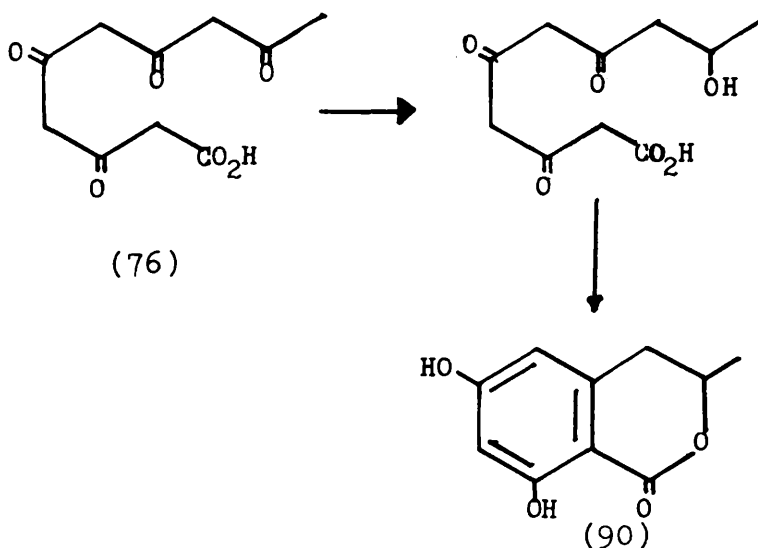
Hill then synthesised the isocoumarin (90) in labelled form and fed it to A. terreus. The terrein isolated was found to have a comparatively high incorporation of radioactivity. Degradation experiments confirmed that dihydroisocoumarin (90) was in fact converted into terrein without any significant degradation to acetate and was thus identified as the

first biosynthetic intermediate on the main pathway to terrein.



Interestingly, it was found that when the isocoumarin (91) was fed to Aspergillus terreus it was not incorporated and consequently does not lie on the pathway to terrein.

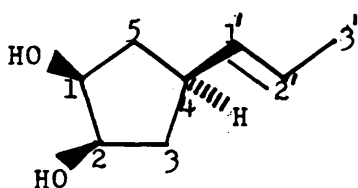
It was proposed that the isocoumarin could be formed by cyclisation of the pentaketide chain (76) as shown below.



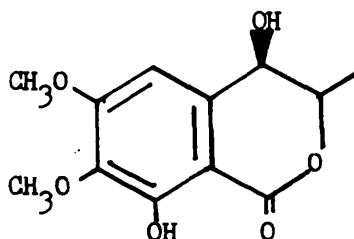
The identification of the isocoumarin (90) as an intermediate on the pathway to terrein is not that

surprising. Recently, further metabolites of the fungus Aspergillus terreus have been identified.<sup>65</sup>

The five-membered ring compound (92) has been isolated from standing cultures of Aspergillus terreus, as has the isocoumarin (93).



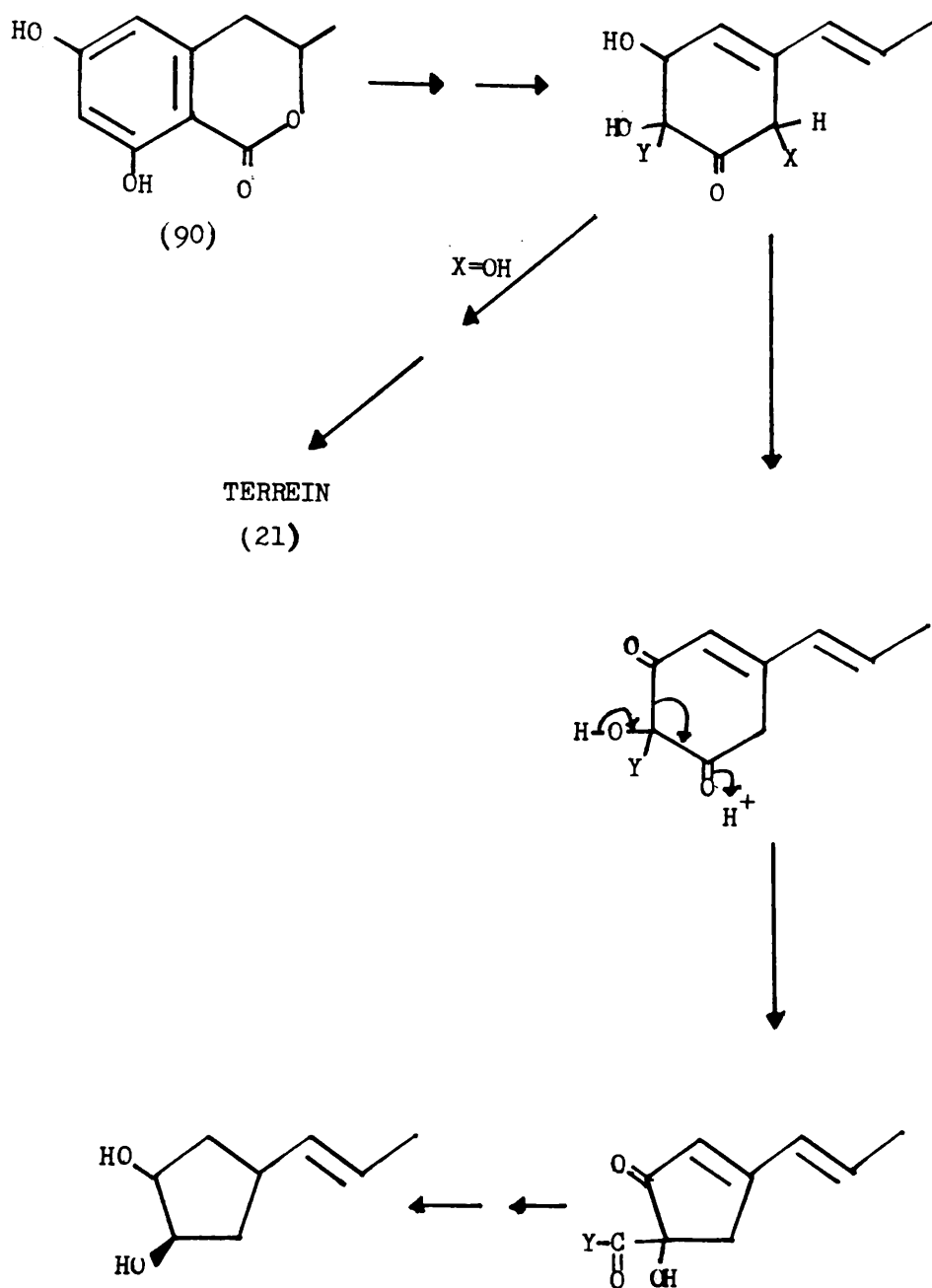
(92)



(93)

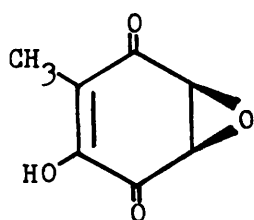
The origin of (92) is difficult to rationalise. It could be a catabolic product of terrein which accumulates in aged cultures. From the biosynthetic evidence already known about terrein it is unlikely that (92) is a precursor of terrein. A third possibility is that (92) may arise from the known precursor, the isocoumarin (90). The proposed scheme 20 reflects the partitioning of the isocoumarin between the two pathways whose relevant importance is a function of the age of the culture.



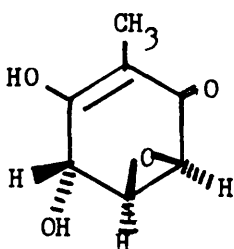


Scheme 20.

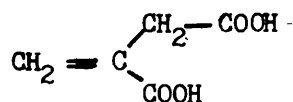
During an investigation by Yamamoto<sup>66</sup> in Japan, further metabolites of Aspergillus terreus were isolated, namely a novel metabolite called "astepyrone" and four other new metabolites. When the culture filtrate was extracted and treated with a 10% sodium bicarbonate solution, Yamamoto obtained the following five compounds which were already known to be produced by strains of Aspergillus terreus: Terreic acid (94), terremutin (95), itaconic acid (96), orsellinic acid (11), and 3,6-dihydroxybenzoquinone (97).



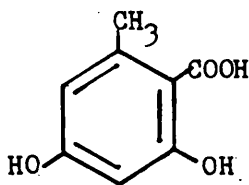
Terreic acid  
(94)



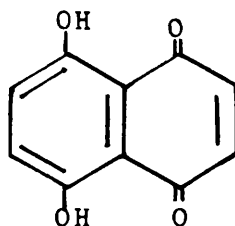
Terremutin  
(95)



Itaconic acid  
(96)



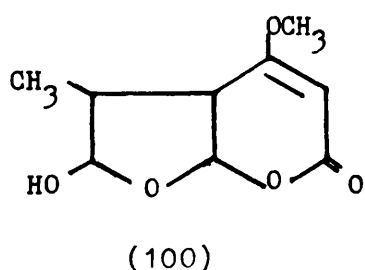
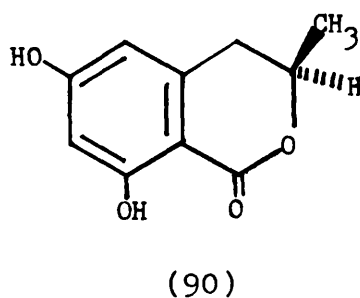
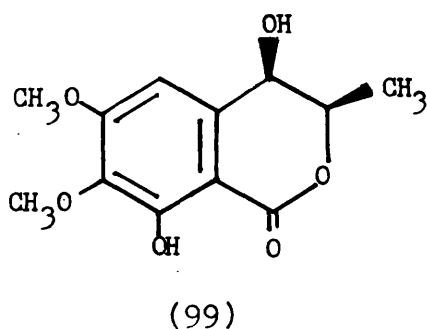
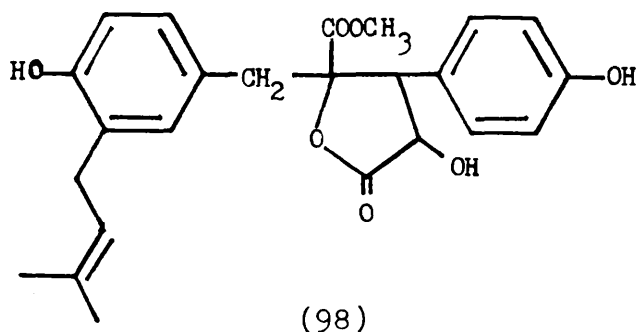
Orsellinic acid  
(11)



3,6-Dihydroxybenzoquinone  
(97)

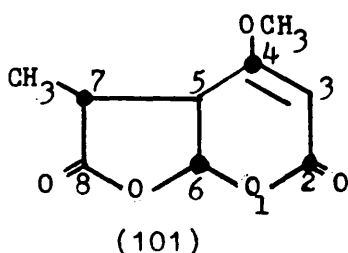
When the culture filtrate was not treated with any bicarbonate solution and only extracted with diethyl ether then they obtained  $\alpha$ -oxo- $\beta$ -(4-hydroxyphenyl)- $\gamma$ -[4-hydroxy-3-(3-methyl-2-buten-1-yl)benzyl]- $\gamma$ -methoxy carbonyl- $\gamma$ -butyrolactone (98) and two other new metabolites corresponding to the diprenyl derivative and the epoxide derivative. Two other isocoumarin

derivatives were also identified; one was a new compound (99) and the other the 3(R)-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (90), the known biosynthetic precursor of terrein.

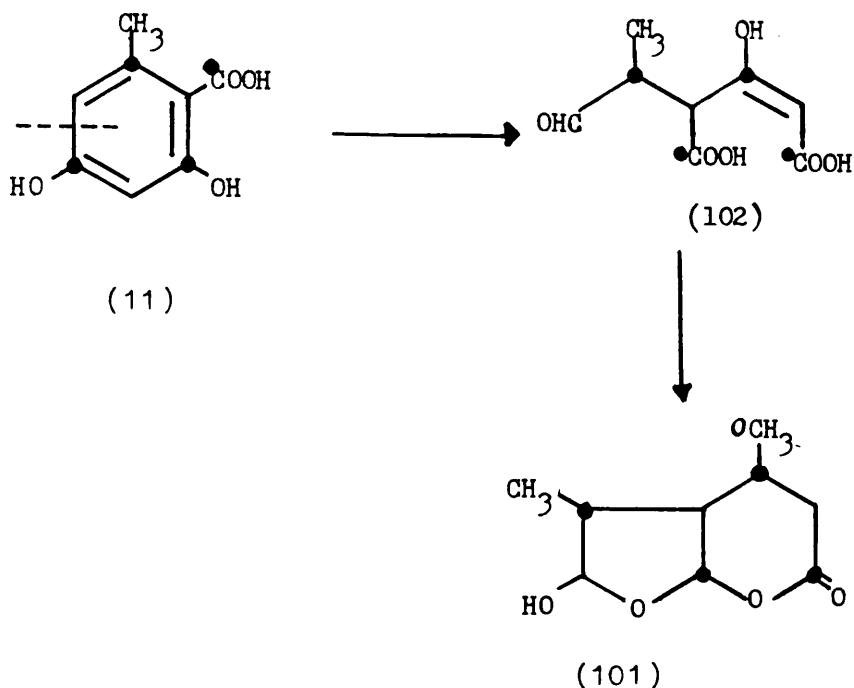


Astepyrone (100) was isolated from the ethyl acetate extract of the culture filtrate along with terrein. Astepyrone is a neutral compound which is unstable in both acidic and even more so in basic media. It is also optically active. Yamamoto

thought that astepyrone (100) may be biosynthesised via the polyketide pathway and to illustrate this he administered  $[1-^{13}\text{C}]$  sodium acetate to the culture medium. The  $^{13}\text{C}$  enriched astepyrone was oxidised to the corresponding lactone (101) to provide a simpler n.m.r. spectrum. Four of the eight carbons were found to be enriched in an alternating manner which showed that astepyrone was indeed of polyketide origin.

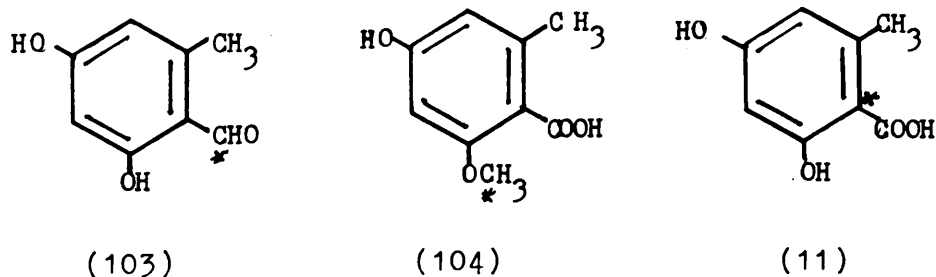


From the structure and distribution of the  $^{13}\text{C}$  labelled carbons in astepyrone (101), it seemed likely that orsellinic acid (11) may be a precursor of (101). If astepyrone was formed by oxidative cleavage of an orsellinic acid derivative at C-4-C-5, then the pattern of  $^{13}\text{C}$  enriched carbons would be expected to coincide with the experimental result obtained. If orsellinic acid is a precursor then astepyrone must be biosynthesised via a rather unstable  $\beta$ -ketoacid (102).



In order to avoid decarboxylation at this step a methoxy group may be introduced before ring opening, thus orsellinic acid-2-methyl ether (104) is a potential precursor. It is also possible that the carbonyl group at C-6 might be derived by way of an aldehyde group, so orsellinic aldehyde (103) might be another attractive precursor to consider.

Yamamoto prepared the two possible orsellinic acid derivative precursors (103) and (104) and orsellinic acid (11) itself in radio-labelled form.



When these  $^{14}\text{C}$  labelled compounds were fed to Aspergillus terreus the results obtained showed that (104) was not incorporated into astepyrone, suggesting that methylation must occur after ring cleavage. (105) and (11) were both incorporated to a high extent into astepyrone, thus both are possible precursors of (100). When radioactive orsellinic acid was fed to the culture broth, the liberated carbon dioxide was radioactive. However, in the case of orsellinic aldehyde (103) the carbon dioxide liberated was found to contain no radioactivity showing that the aldehyde was not converted to the acid. From these results Yamamoto concluded that orsellinic aldehyde (103) was a more closely related intermediate than orsellinic acid (11) and proposed the biosynthetic route shown in scheme 21.



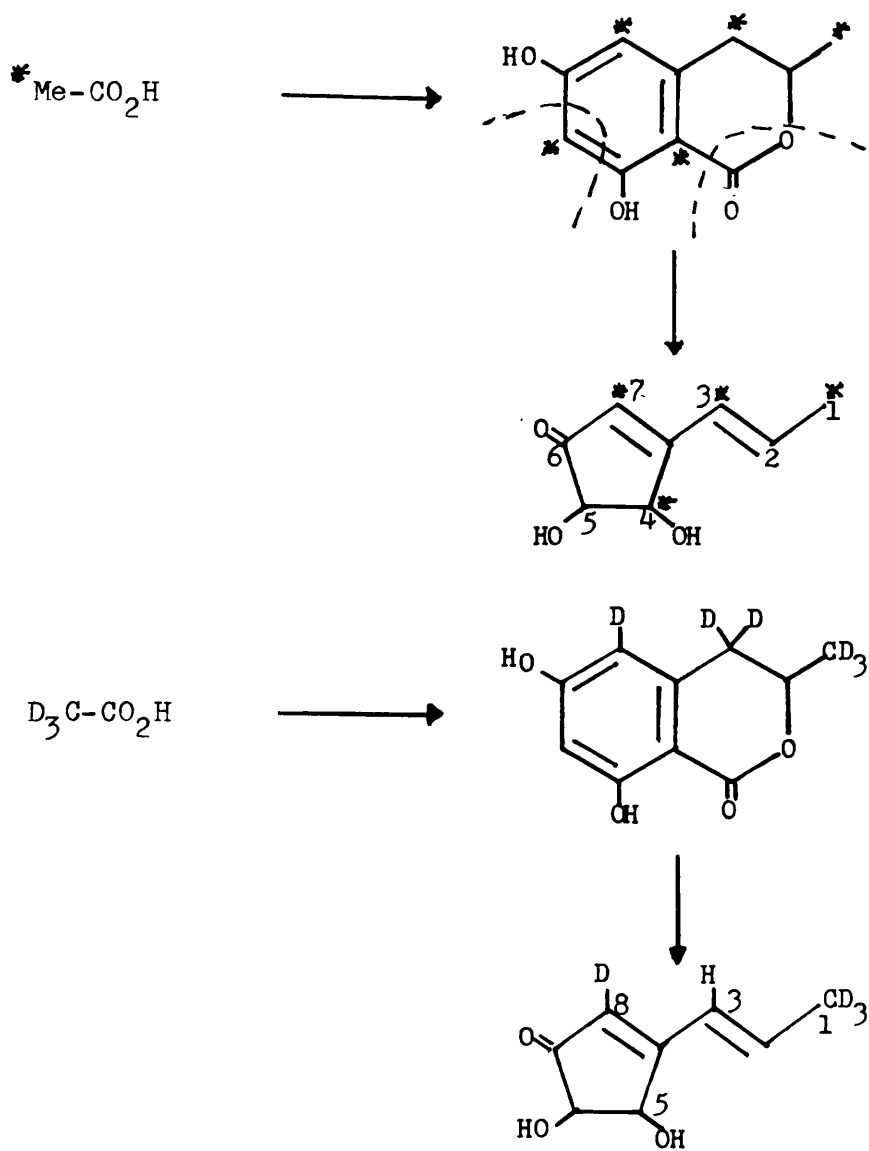
In later studies on the biosynthesis of terrein, Staunton<sup>67</sup> et al used [ 2-<sup>13</sup>C, 2-<sup>2</sup>H<sub>3</sub> ] acetate to investigate the retention of hydrogen from the methyl group of acetate. As previously mentioned, the detection of deuterium through its coupling to carbon-13 in a <sup>13</sup>C n.m.r. spectrum of multiply labelled molecules offers the possibility of establishing the integrity of C-H bonds during the course of a biosynthetic pathway.

Staunton used three different precursors [ 1-<sup>14</sup>C ] acetate, [ 2-<sup>13</sup>C, 1-<sup>14</sup>C, 2-<sup>2</sup>H<sub>3</sub> ] acetate and [ 2-<sup>14</sup>C ] acetate mixed with [ 2-<sup>13</sup>C, 1-<sup>14</sup>C, 2-<sup>2</sup>H<sub>3</sub> ]-acetate. In principle three out of the four methyl group-derived carbons of terrein could retain deuterium from [ 2-<sup>13</sup>C, 2-<sup>2</sup>H<sub>3</sub> ] acetate as seen in scheme 22. The hydrogen at C-5 must be derived from the medium and so this carbon atom can be used as an internal standard. It is also possible that some loss of deuterium from other carbons may take place by exchange at one or more of the intermediate stages of the biosynthesis. In using deuteriated acetate as a precursor, there is a risk that isotope effects may alter the efficiency of incorporation of acetate into the chain-building units to such an extent that there is non-uniform labelling of the polyketide chain. This possibility was checked by incorporation studies with [ 1-<sup>14</sup>C, 2-<sup>13</sup>C, 2-<sup>2</sup>H<sub>3</sub> ] acetate and the results showed that deuteriated acetate is incorporated



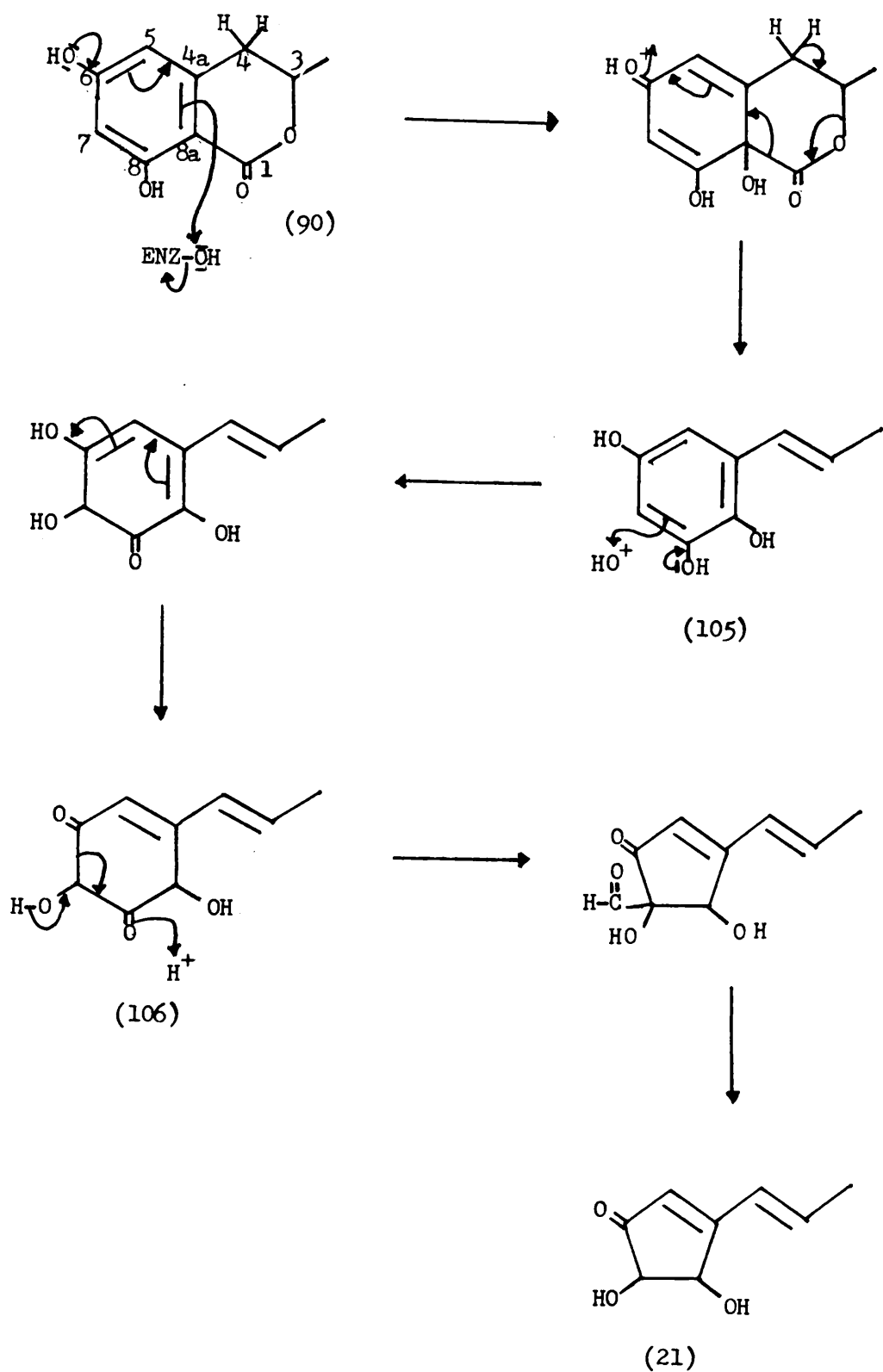
in the same way as proteo-acetate within experimental error.

The results of feedings with  $[2-^{13}\text{C}, 2-^2\text{H}_3]$ -acetate showed that C-1, C-3, C-5 and C-8 were intense signals in the  $^{13}\text{C}$  n.m.r. spectrum of terrein. Thus proving that  $^{13}\text{C}$  had been incorporated at these positions. Comparison of the intensity of the C-6 unenriched signal with the C-5 signal (which cannot retain deuterium) showed that the latter was enriched five-fold relative to natural abundance. The signals from C-1, C-3 and C-8 were of lower intensity, consistent with the presence of deuterium at these positions. However the  $^{13}\text{C}$ -D triplet which should correspond to C-3 was not seen. This means that either no deuterium is present at C-3 or the triplet must be much less intense than that for C-8. Staunton concluded that this may be a result of longer relaxation time for C-3 rather than a greater degree of exchange at that position during biosynthesis.



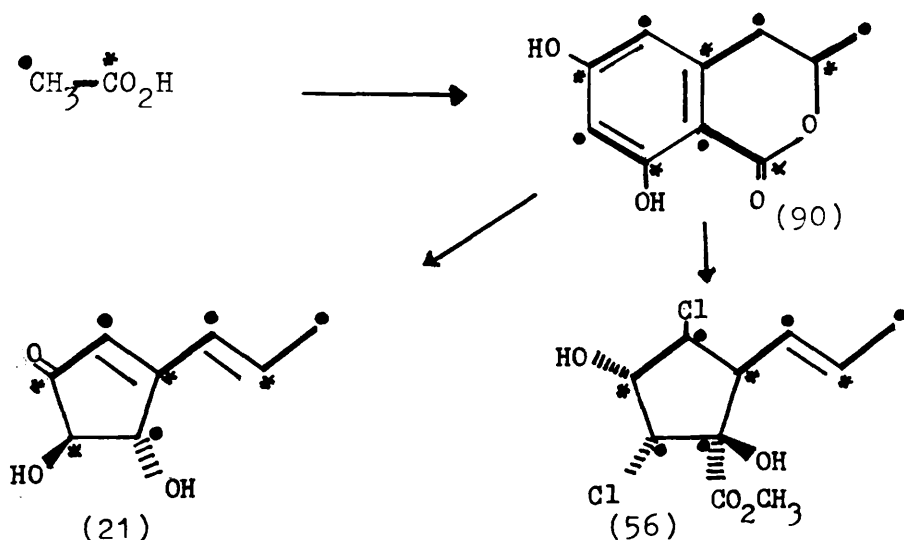
Scheme 22.

It has previously been shown that the isocoumarin (90) is a true metabolic intermediate on the pathway to terrein. However, what is not known is how this intermediate proceeds from this point to go on and form terrein. Labelling studies have shown that a ring contraction must be involved with C-7 of the isocoumarin being extruded. Also known is that hydroxylation of the ring is required as well as loss of the carboxyl carbon C-1. Based on the evidence already known about terrein biosynthesis, a possible biosynthetic route from the isocoumarin (90) could be put forward (Scheme 23.) This route involves enzymic oxidation of carbon-8a of the isocoumarin with subsequent loss of C-1 as carbon dioxide leading to an intermediate such as (105). Hydroxylation of the ring at C-7 followed by tautomerism would produce the quinone (106) which could then undergo a ring contraction with loss of C-7 to give terrein.



Scheme 23.

Henderson<sup>47</sup> carried out analagous studies on the biosynthesis of cryptosporiopsinol (53), a compound structurally similar to terrein. The isocoumarin (90) was found also to be a precursor of this compound. It is therefore not unreasonable to assume that since both terrein (21) and cryptosporiopsinol (56) have the isocoumarin (90) as a common intermediate and are both derived by way of a polyketide type pathway, then they may well have other common intermediates.



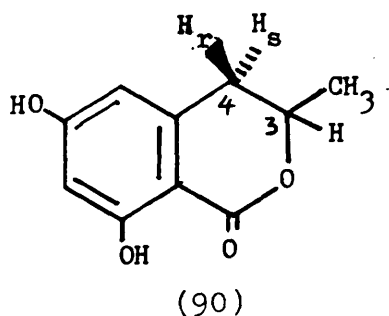
It is known however, that although both compounds are formed by way of a ring contraction, the carbon which is extruded is different in each case. In terrein it is C-7 of the isocoumarin which is lost as can be seen from the labelling pattern, while in cryptosporiopsinol it is C-8 of the isocoumarin which is lost. Thus the latter stages of the biosynthesis of these two compounds must at least be different.

At the start of this project, it was decided that

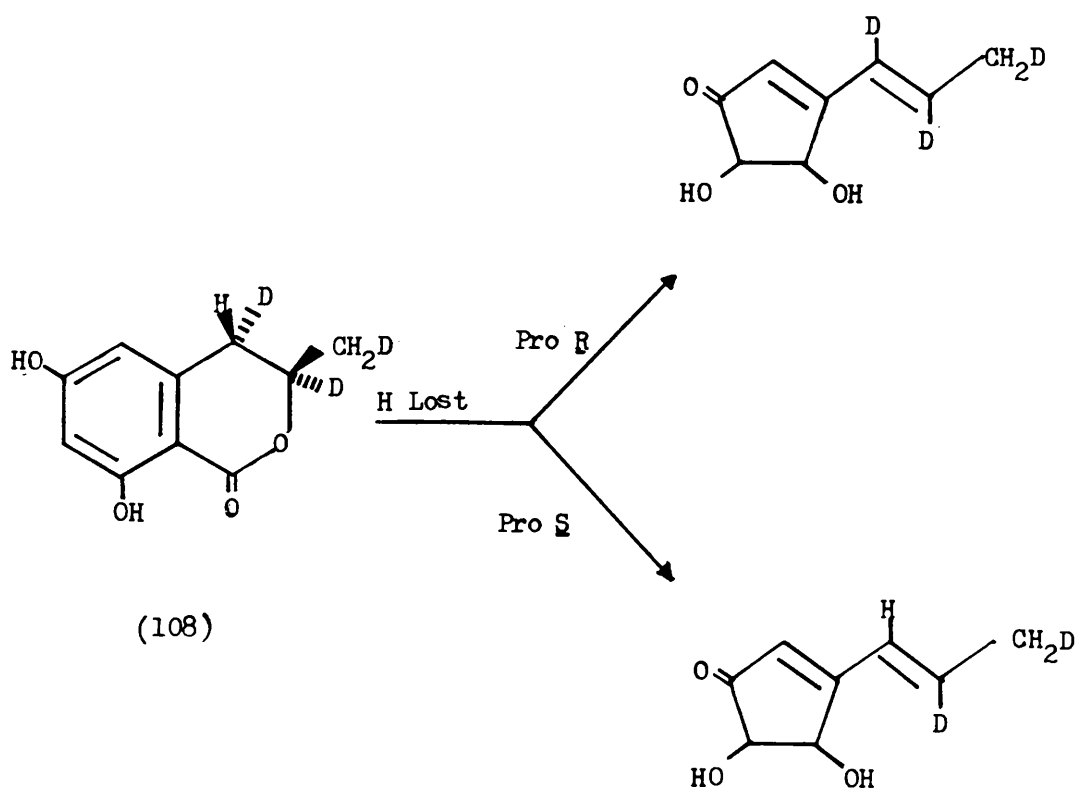
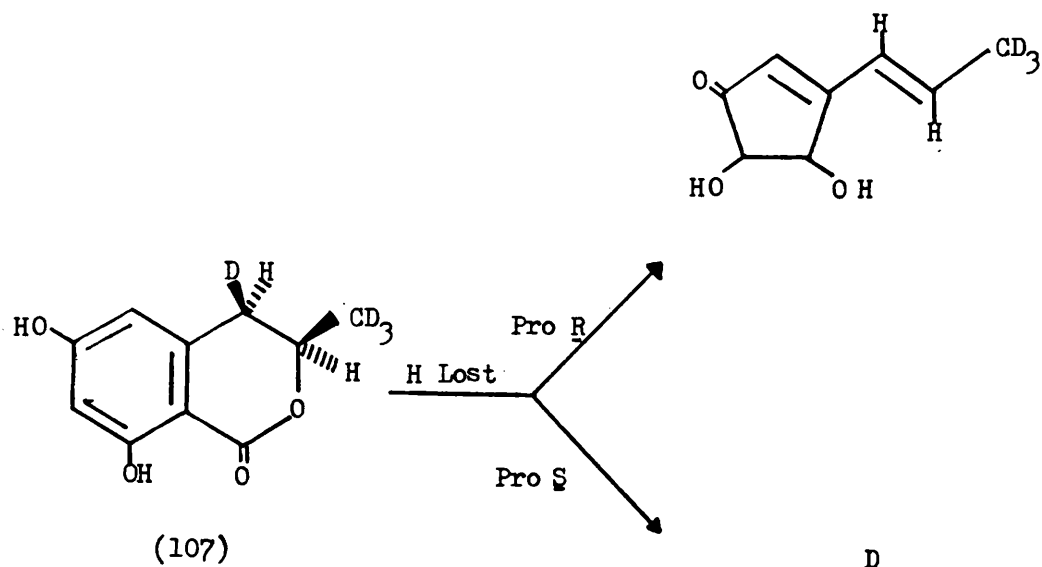
more information was needed regarding the nature of the ring cleavage step in the biosynthesis of terrein. If more possible intermediates on the pathway to terrein could be identified, especially those involved in the latter stages of the biosynthesis then this might give a better understanding as to the mechanism of the ring contraction. Clearly it would also be beneficial if some knowledge regarding the nature of the side chain formation in terrein could be obtained.

In order to investigate the mechanism by which the propenyl group in terrein is formed, the stereochemistry of the elimination process was investigated. Since the isocoumarin (90) is a known intermediate it would be beneficial to use this compound in our studies. The fate of the three protons at positions three and four of the dihydroisocoumarin needed to be traced.

The stereochemistry of the propenyl group formation could be determined if it was known which of the prochiral hydrogens at C-4 of the dihydroisocoumarin is lost (assuming the process is indeed stereospecific). It would also be interesting to note whether the hydrogen at C-3 is retained throughout the biosynthesis.



To follow the fate of these three hydrogens at C-3 and C-4 a synthesis of two differently deuterium labelled isocoumarins (107) and (108) was needed. These two compounds would enable the identification of the hydrogen that is lost at C-4 of the isocoumarin in the course of the biosynthesis. If the hydrogen is lost from the pro R position, then on feeding the two labelled isocoumarins to Aspergillus terreus, the terrein isolated should contain a deuterium label at C-3 of terrein in the case of (107) and no label when fed with (108). Similarly if it is the pro S hydrogen that is lost, then there would be no deuterium label at C-3 when fed with (107) but there would be a deuterium at C-3 when (108) is fed. The fate of the proton at C-3 of the isocoumarin can easily be followed. If the hydrogen at this position is retained throughout the biosynthesis then a deuterium will be present at C-2 of terrein when fed with (108) but a hydrogen when (107) is fed.





A route to the [3-<sup>2</sup>H]-[4-<sup>2</sup>H pro R] isocoumarin (107) and the [4-<sup>2</sup>H pro S] isocoumarin (108) was achieved successfully. These could then be fed in racemic form and as their sodium salts. It is known that the fungus will only incorporate the 3-R form of the dihydroisocoumarin so it was not significant that the isocoumarins were fed as racemates. Consequently the dihydroisocoumarins are incorporated with either 4R or 4S configurations.

Before any feedings of the two labelled isocoumarins (107) and (108) were carried out, a time-study on Aspergillus terreus was needed. This was to determine the optimum length of time to allow the fungus to grow thus providing the maximum amount of terrein extract possible.

#### Aspergillus terreus Time Study

<u>No. of Days</u>	<u>Crude Extract Weight (mg)</u>
7 (Ext 1)	127
7 (Ext 2)	121
14 (Ext 1)	603
14 (Ext 2)	179
20	95

From the results seen in the table above, it was concluded that the greatest amount of terrein was obtained from the terrein extract which had been cropped off after 14 days.

The isocoumarins (107) and (108) were fed to Aspergillus terreus. The first feeding produced virtually no terrein at all, the two labelled isocoumarins being recovered intact from the culture medium. The reason for the failure of the fungus to produce any terrein could have been due to any number of reasons. Fungi are very sensitive to any small changes that might occur either in the concentrations of nutrients in the culture medium or variations in temperature and pH. Thus any discrepancies in these variables could halt the production of terrein.

A second batch of feedings were prepared. However, this time the culture, before any feedings took place, did not visually appear as it normally should. The mycelium was extremely thin and any movement disrupted this top layer; consequently this second attempt was abandoned. A report in the literature, recently revealed that Aspergillus terreus grew better and produced greater quantities of terrein when subjected to increased light. For the third attempt a surface culture of Aspergillus terreus was grown under extra light. The mycelium appeared healthy and the two labelled isocoumarins (107) and (108) were refed to the culture medium as their sodium salts on day 7.

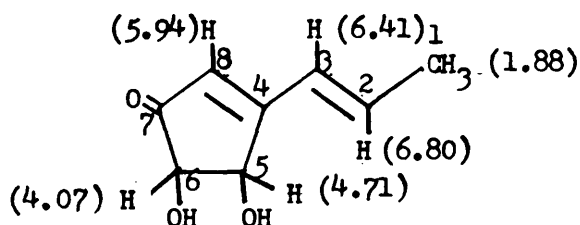
The terrein isolated from the two feedings was analysed spectroscopically using  $^{13}\text{C}$  and  $^2\text{H}$  n.m.r. The presence of deuterium in a molecule will affect its

$^{13}\text{C}$  n.m.r. spectrum in two major ways:

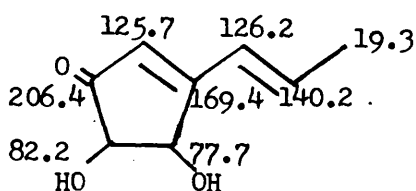
1. In the proton noise decoupled spectrum the signal due to the carbon directly bonded to the isotope will be seen as a triplet of lower intensity.
2. The carbon directly bonded to the isotope and those on either side will experience small downfield shifts.

In order to draw any conclusions from the feedings of these deuterioisocoumarins, an unambiguous assignment of all the signals in the proton n.m.r. and carbon-13 n.m.r. of unlabelled terrein is required. A pure sample of terrein was isolated from Aspergillus terreus during the time study and a  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r obtained for this compound. The sample was recrystallised twice from acetone as pale yellow needles then dissolved in deuterioacetone and a proton n.m.r. obtained. The assignment of peaks in the proton n.m.r. are as follows. The signal due to the methyl group is a doublet at  $\delta$  1.88 with coupling constant  $\underline{J} = 7$  Hz. The signal from  $\text{H}_2$  is a double quartet centred at  $\delta$  6.80 with coupling constants of 7 and 16 Hz. The signal from  $\text{H}_3$  is seen as a doublet at  $\delta$  6.41 with coupling constant  $\underline{J} = 16$  Hz. The broad singlet at  $\delta$  5.94 is due to  $\text{H}_8$ . The signals for  $\text{H}_5$  and  $\text{H}_6$  appear at  $\delta$  4.71 and  $\delta$  4.07 respectively. The peak at  $\delta$  4.07 shows a fine splitting of 2 Hz where as the peak at  $\delta$  4.71 is broad and unresolved, this is

probably due to the allylic coupling between  $H_8$  and  $H_5$ . Hill<sup>64</sup> had previously come across this feature in his work. He showed that irradiation of  $H_8$  caused sharpening of the signal at  $\delta$  4.71 to give a doublet split by 2 Hz while the other signal at  $\delta$  4.07 remained the same. Thus, the doublet at  $\delta$  4.71 can be assigned to  $H_5$  and the signal at  $\delta$  4.07 to  $H_6$ . This assignment is unusual in that the proton nearest to the carbonyl group has a lower chemical shift value than the next carbon. Clearly a very complex system is involved here.



An assignment of all the lines in the  $^{13}\text{C}$  n.m.r. spectrum was also required. The line at 19.3 ppm is a quartet and due to the methyl group in terrein. Carbons 2 and 3 are both seen as doublets at 140.2 and 126.2 ppm respectively. Carbon 4 is seen as a singlet at 169.4 ppm. Carbons 5 and 6 are both doublets at 77.7 and 82.2 ppm respectively. The singlet at 206.4 ppm is due to the carbonyl carbon C-7. Finally, carbon 8 can be seen as a doublet at 125.7 ppm.



(21)

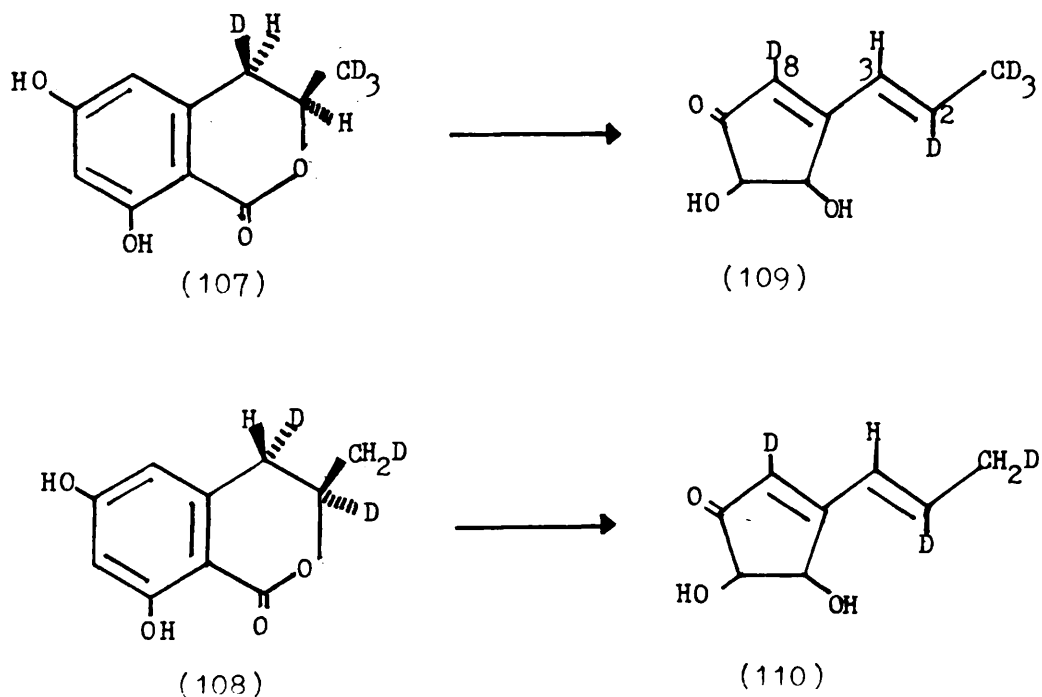
With all the peaks in the proton and carbon-13 n.m.r. spectrums now assigned, a detailed analysis of the terrein isolated from the feeding of the two labelled isocoumarins can now be carried out.

The terrein isolated from Aspergillus terreus when the deuterioisocoumarin (107) was fed, was analysed using deuterium and carbon-13 n.m.r. The  $^2\text{H}$  n.m.r. showed broad signals at  $\delta$  6.78 and  $\delta$  5.94 indicating the presence of deuterium at C-2 and C-8 respectively. A large peak at  $\delta$  1.83 is due to the  $-\text{CD}_3$  grouping in terrein. The incorporation level was not sufficiently high enough for any  $\beta$ -shifts to be detected in the  $^{13}\text{C}$  n.m.r. spectrum. However it could be seen that the sample was indeed pure.

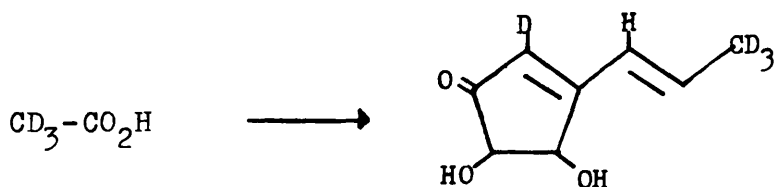
Analysis of the  $^2\text{H}$  n.m.r. spectrum of terrein obtained when the deuterioisocoumarin (108) was fed to Aspergillus terreus showed two broad signals at  $\delta$  6.78 and  $\delta$  5.94 indicating that a deuterium is present at C-2 and C-8 of terrein. A small singlet at  $\delta$  1.84 was noted, this being due to a small amount of deuterium present in the methyl group of terrein. Again the

$^{13}\text{C}$  n.m.r. spectrum did not show any  $\beta$ -shifts because of the low incorporation level.

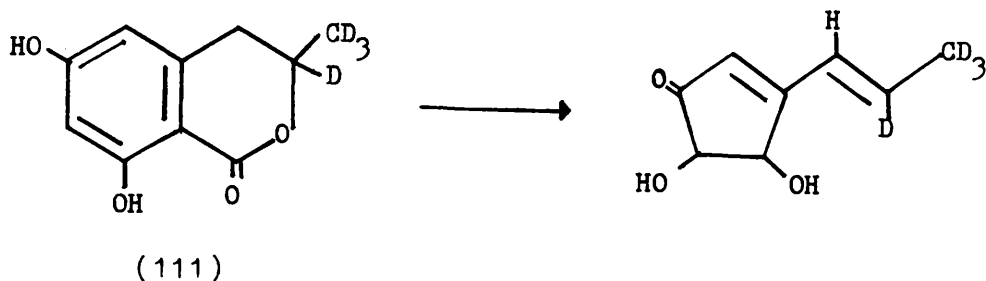
From the feeding results it can be concluded that deuterium is present at C-2 and C-8 in both samples of terrein isolated after feeding with the two deuterioisocoumarins (107) and (108). The relative intensities of the lines responsible for C-2 and C-8 in terrein (109) were found to be approximately in the same ratio as the lines observed in the terrein sample (110). It was also noted that a great deal more deuterium is present in the methyl group of terrein (109) than in the terrein sample (110). These results indicate that deuterium from the two isocoumarins (107) and (108) is being incorporated in the following manner:-



These results indicate that at least some partial degradation of the two isocoumarins (107) and (108) to acetate must be occurring. This would account for the presence of deuterium at C-8 in both terrein samples. However at least some of the two isocoumarins must be incorporated intact since deuterium is seen at C-2 in both samples of terrein isolated from the two feedings. This is what would be expected if an intact isocoumarin deuteriated at C-3 was incorporated. Staunton<sup>67</sup> has previously shown that feedings with  $[2-^{13}\text{C}, 2-^2\text{H}_3]$  acetate produced terrein which had a deuterium label at C-1 and C-8. No deuterium was found at C-3 of terrein in either of the samples, although deuterium was present in the 4-position of both isocoumarins fed. Thus the deuterium at this position must be lost at some undefined stage.



The synthetic route leading to the isocoumarin (107) also produces a little of the isocoumarin (111), which means that the sample of the isocoumarin (107) may be contaminated with some of the isocoumarin (111). This would account for the presence of deuterium at C-2 when (107) is fed to Aspergillus terreus.



Some of the isocoumarin (107) is indeed incorporated intact into terrein. There is no sign of any deuterium present at C-3 of (109) thus it can be concluded that this must have been lost after dihydroisocoumarin formation. In the case of the isocoumarin (108) some has been degraded to acetate but some has also been incorporated intact. The deuterium at C-3 of (108) has been shown to be retained throughout the biosynthesis thus showing that a proton at C-3 of the dihydroisocoumarin (90) may well be retained throughout the biosynthesis.

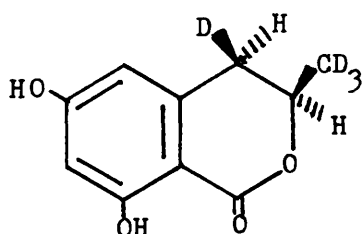


### Chapter 3A.

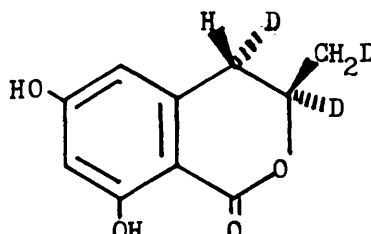
#### Synthesis of Labelled Isocoumarins.

##### A route to deuterium labelled 6,8-dihydroxy-3-methylisocoumarins.

As mentioned in the previous chapter the main target was to find a synthetic route to the two deuterium labelled isocoumarins (107) and (108).



(107)



(108)

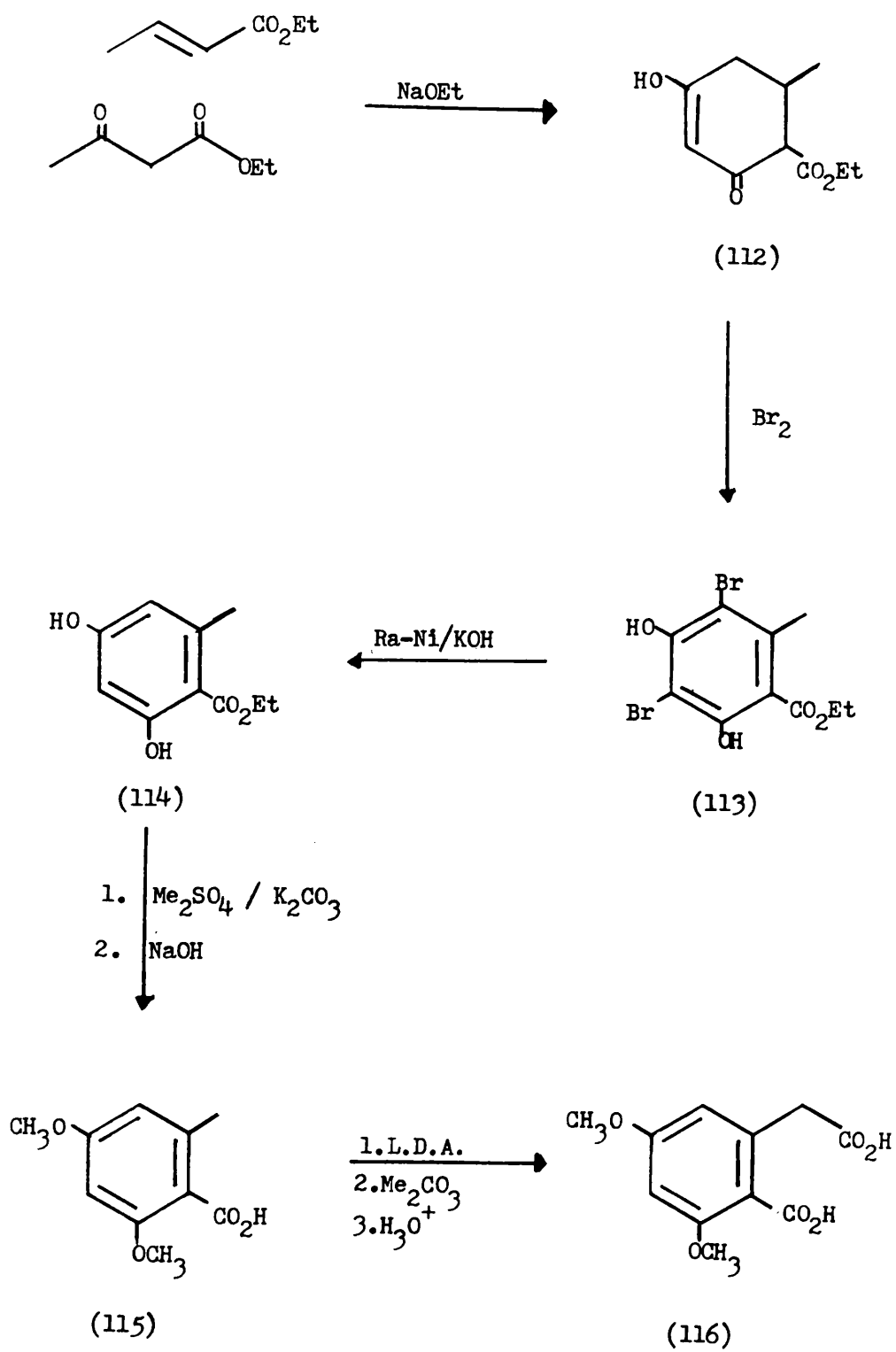
If these compounds could be obtained then they could be fed to Aspergillus terreus and the terrein produced analysed spectroscopically for the presence of deuterium. This would allow more information on the biosynthesis of terrein to be gained.

There are now hundreds of examples of both synthetic and natural isocoumarins reported in the literature. Due to their large numbers and diverse structures a large variety of synthetic routes were available to choose from.<sup>68</sup> The initial approach was to try and synthesise the undeuteriated isocoumarin (122) and then attempt to label this isocoumarin with deuterium at the required positions giving the deuterioisocoumarins (107) and (108). After an extensive literature search it was decided that an approach via the homophthalic acid derivative (116) would be the best route. The

synthesis was relatively short and all products looked as though they could be obtained easily and in good yield.

A route to the homophthalic acid (116) was thus needed. The first approach employed was one that Henderson<sup>47</sup> had used. (Scheme 24) Ethyl acetoacetate was condensed with ethyl crotonate to produce ethyl 1,2-dihydro-o-orsellinate (112), this was then aromatised with bromine to the dibromo compound (113). Debromination of (113) with Raney nickel in sodium hydroxide should then afford ethyl-o-orsellinate (114). However on attempting this reaction only a black tar-like substance was produced which was not able to be sufficiently purified to be used in the next stage. The reaction was repeated several times but only a very small amount of the pure ethyl-o-orsellinate was ever obtained. In the light of this, attempts to make the homophthalic acid by this method were abandoned.

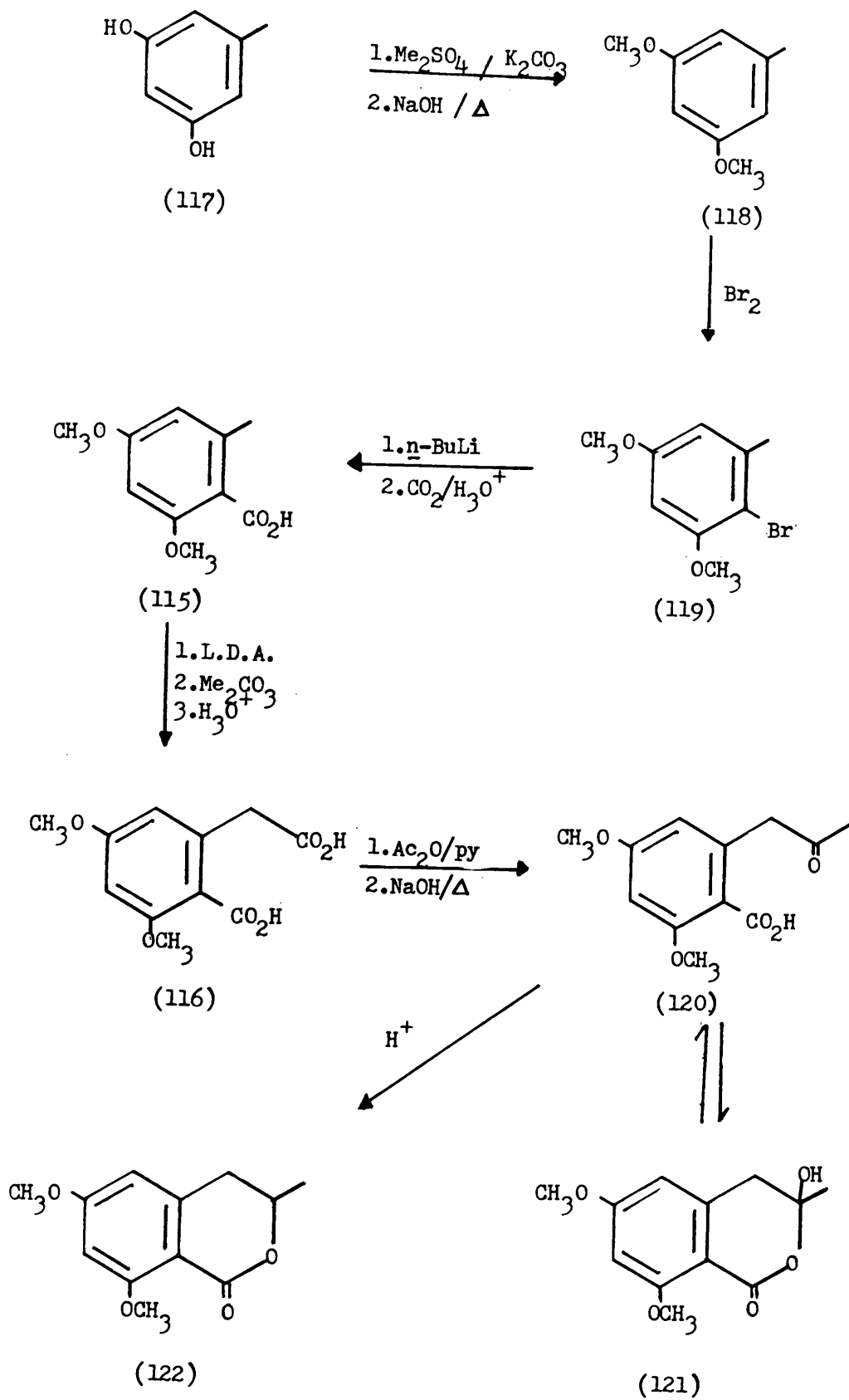
It was then decided that a better approach would be via a route devised by McAuley.<sup>69</sup> (Scheme 25) The starting point was from the commercially available orcinol monohydrate. Orcinol (117) was methylated in good yield using dimethyl sulphate to give the dimethoxy compound (118). Treatment of this with 1 mole of bromine in 1,2-dichloroethane afforded the 2-bromo-derivative (119) in good yield. Small amounts of the 2,6-dibromo-derivative were easily removed by crystallisation. The monobromo compound (119) was



Scheme 24

then treated with n-butyllithium to give a lithiated derivative which was quenched with carbon dioxide. On acidification with dilute acid 2,4-dimethoxy-6-methyl benzoic acid (115) was obtained. The dianion produced by the action of lithium diisopropylamide on the acid (115) was then carboxylated with dimethylcarbonate to give the required homophthalic acid derivative (116).

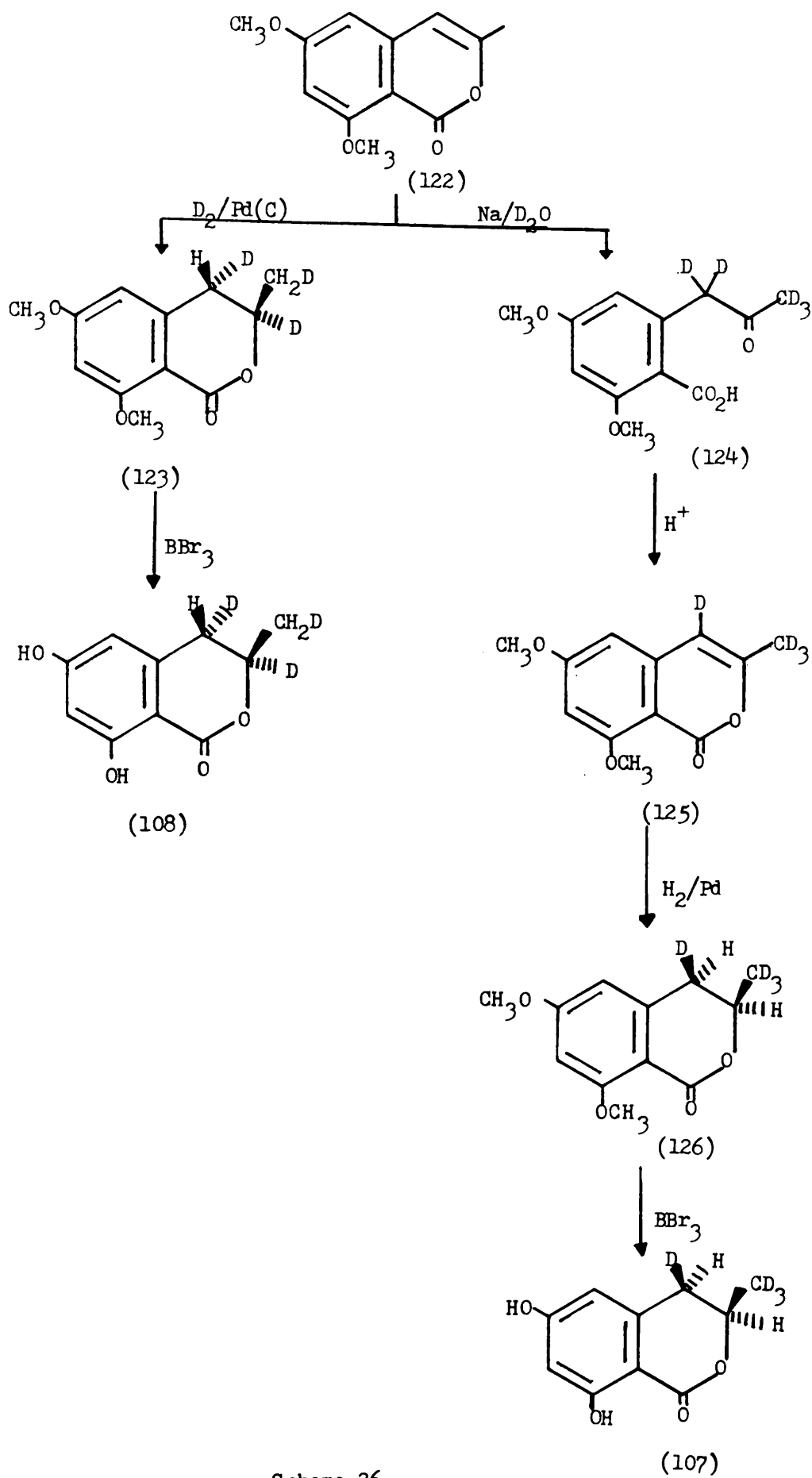
There are many known routes in which homophthalic acids are converted into isocoumarins.<sup>68</sup> The best route was thought to be via acetylation of the homophthalic acid. 3,5-Dimethoxyhomophthalic acid (116) was treated with a mixture of acetic anhydride and pyridine at room temperature to give an intermediate which was not isolated.<sup>70</sup> This was then refluxed in a 4M sodium hydroxide solution to give the oxopropyl compound (120). It is thought that (120) actually exists in the lactone form (121) due to infra-red data which shows the presence of only one carbonyl group. There is also very little indication of any characteristically strong, hydrogen bonded OH, normally present in a carboxylic acid. The keto-acid (120) was converted into the isocoumarin (122) by the method of Edwards and Rao.<sup>71</sup> This involved standing (120) in a solution of ethyl acetate, acetic anhydride and perchloric acid for ten minutes, after which time an acid catalysed ring closure had been achieved giving the isocoumarin (122) in good yield.



Scheme 25

This isocoumarin (122) can now be used as the starting point for the production of the two required deuterioisocoumarins (107) and (108). Scheme 26

Deuterium was introduced into the isocoumarin by two completely different procedures so that the correct stereochemistry of the two required isocoumarins would be achieved. The deuterioisocoumarin (107) was obtained by stirring (124) with sodium deuterioxide. All the acidic protons present in the molecule were exchanged for deuterium, as were the three protons of the methyl group. This pentadeuterio compound (124) can then be cyclised to the deuterioisocoumarin (125) as before using the method of Edwards and Rao. Hydrogenation using hydrogen gas on a palladium/carbon catalyst afforded the dihydroisocoumarin (126) with the correct stereochemistry. Removal of the methoxy protecting groups was successfully achieved by demethylating with boron tribromide. Thus the first deuterioisocoumarin (107) which was required was successfully synthesised with the labels in the correct positions. The other deuterioisocoumarin (108) required was synthesised by treating the isocoumarin (122) with deuterium gas on a palladium/carbon catalyst. The deuteriated isocoumarin (123) obtained now had deuteriums which were cis to each other. Demethylation of (125) with boron tribromide afforded the second target isocoumarin (108) with the correct stereochemistry.



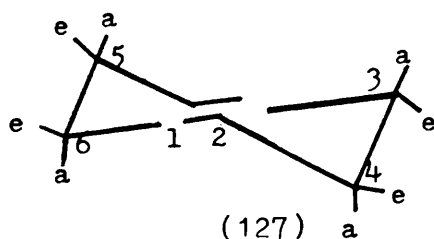
Scheme 26

These two isocoumarins could now be fed as their sodium salts to Aspergillus terreus and their incorporation monitored.

### Confirmation of Structure

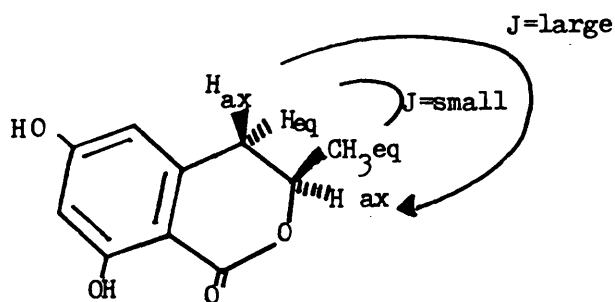
With the synthesis of the two deuterio-labelled isocoumarins (107) and (108) now completed, it was necessary to fully characterise them. Confirmation that the deuterium atoms were indeed at the correct positions in the two isocoumarins was required. This was done primarily by using spectroscopic techniques, especially carbon-13 and deuterium n.m.r.

When a cyclohexane ring contains trigonal carbon atoms, its normal chair shape is distorted. Consider cyclohexene (127), it can be seen that the carbons of the ethylene group and the two adjacent allylic carbons (3 and 6) are all in a plane. However, the atoms on the other side (carbons 4 and 5) are normally staggered as in cyclohexane. The hydrogen (or other) atoms attached to carbons 4 and 5 normally occupy equatorial and axial positions. Those atoms attached to carbons 3 and 6 are imperfectly staggered and do not take up the normal equatorial and axial positions. These atoms are described as occupying pseudo-equatorial and pseudo-axial positions.



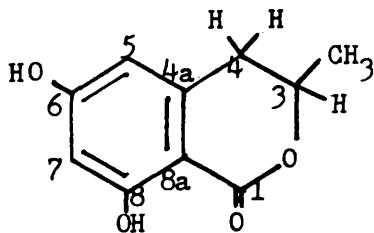


The six-membered ring in (90) is not a true cyclohexane shape due to the presence of the  $sp^2$  hybridised carbons. Any substituents at the 3 or 4 position will occupy pseudo-equatorial and pseudo-axial sites. The methyl group at C-3 is the only substituent and because of its bulkiness, will occupy a pseudo-equatorial position. A substituent which is cis to the methyl group will occupy a pseudo-axial site and a substituent which is trans to the methyl group will occupy a pseudo-equatorial position.



(90)

Analysis of the  $^1\text{H}$  n.m.r. of 3,4-dihydro-6,8-dihydroxy-3-methyl isocoumarin (90).



(90)

In order to fully characterise the two deuterio-isocoumarins (107) and (108), all the signals in the  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectrum of the unlabelled isocoumarin

(90) needed to be identified. The  $^1\text{H}$  n.m.r. spectrum of (90) shows a multiplet centred at  $\delta$  6.27 which is due to the two aromatic protons at C-5 and C-7.

A double doublet of quartets can be seen with its centre at  $\delta$  4.67. This is due to the pseudo-axial proton at C-3 which is being split into a quartet by the neighbouring methyl group protons with a coupling constant of 6 Hz. The proton at C-3 is also split by the pseudo-equatorial and pseudo-axial protons at C-4 of the isocoumarin, both with different coupling constants. The two protons at C-4 appear as a doublet of double doublets in the range  $\delta$  3.00 -  $\delta$  2.70. On expansion it can be seen that the pseudo-axial and pseudo-equatorial protons at C-4 are split by each other with a very large coupling constant of 17 Hz. Each of these protons are then split by the pseudo-axial proton at C-3. The pseudo-axial - pseudo-axial splitting has a relatively large coupling constant of 12 Hz while the pseudo-equatorial - pseudo-axial coupling constant is small and equal to 4 Hz. Finally there is a doublet at  $\delta$  1.43 with a coupling constant of 6 Hz due to the methyl group in the isocoumarin.

It is therefore easy to determine which proton has been replaced by deuterium in the isocoumarin. A large coupling constant will be seen if the pseudo-equatorial proton is replaced and a small coupling constant if it is the pseudo-axial proton. This is therefore an excellent method of determining whether the

synthesised isocoumarin has the correct stereochemistry.

Analysis of the  $^{13}\text{C}$  n.m.r. of (90)

The  $^{13}\text{C}$  n.m.r. spectrum of the undeuteriated isocoumarin (90) was obtained and the results are tabulated below.

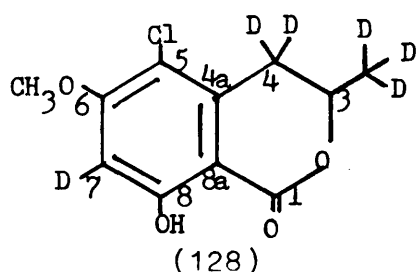
Chemical Shift	( ppm)	Multiplicity	Carbon No.
170.12		s	C=O
164.90		s	C-6 and C-8
143.16		s	C-4a and C-8a
107.28		d	C-7
101.72		d	C-5
76.22		d	C-3
34.95		t	C-4
20.75		q	$\text{CH}_3$

Deuterium Isotope effects on  $^{13}\text{C}$  nuclear shielding.

Isotope effects have been known for a great length of time and have been reviewed on many occasions.<sup>72-77</sup> Batiz-Hernandez<sup>78</sup> and Bernhein came to the conclusion that the magnitude of the isotope shift is generally dependent on how remote the isotope substitution is from the nucleus under observation. The magnitude of the shift is also a function of the shielding range of the resonant nucleus (the isotope shift is largest where the functional change in mass upon substitution is largest) and in general the isotope nuclear shielding is approximately proportional to the number of atoms in the molecule which have been substituted by isotopes.

Deuterium isotope effects are by far the best documented. McAuley<sup>69</sup> carried out an investigation of the isotope effects involved in the isocoumarin (128). The signals for carbons 3, 4a and 8 were seen to be shifted upfield in the  $^{13}\text{C}$  n.m.r. spectrum, this being due to the presence of  $\beta$ -deuterium atoms. These shifts are known as  $\beta$ -shifts and for C-3 have the values of 0.04, 0.08 and 0.12 ppm. This indicates that one, two and three deuterium atoms are present at the methyl group. C-8 also showed a  $\beta$ -shift of 0.04 which was due to the deuterium at C-7. Interestingly, the  $\beta$ -shifts observed for C-4a had values of 0.03, 0.07 and 0.10 ppm, suggesting that the deuterium in the axial position was producing one shift while the equatorial deuterium

was producing another. The third shift of 0.10 ppm is the additive value of the other two shifts and is seen when there are two deuterium atoms at C-4.



<u>Carbon No.</u>	<u><math>\beta</math>-shift</u>
3	0.04, 0.08, 0.12
4a	0.03, 0.07, 0.10
8	0.04

This system could be used as an analogy for the two deuteriated isocoumarins which have been synthesised in attempts to look for deuterium shifts.

Isotope effects can occur over one, two or three bonds, giving rise to  $\alpha$ ,  $\beta$  and  $\gamma$  shifts respectively.<sup>79</sup> Shifts can be observed over more than three bonds, but this does not occur very often. The size of the isotope effect depends on the hybridisation of atoms (i.e.  $sp^2$  or  $sp^3$ ) involved at the carbon bearing the deuterium and the observed carbon.

The following systems show  $\alpha$ ,  $\beta$  and  $\gamma$  isotope effects:-

#### $Csp^3D$ $Csp^3$ effects

##### $\alpha$ -shifts

The isotope effects observed in  $CH_3D$  deuteriated methanes show a shielding change of + 0.187 ppm per deuterium, the effects are largely additive.<sup>80</sup> For

straight chain  $-\text{CD}_2-$  groups an effect of 0.36 ppm per deuterium is observed.<sup>81</sup> Slightly lower values have been observed in the case of cyclohexane.<sup>82,83</sup> These also show a small difference between a deuterium in an axial position and a deuterium in an equatorial position. When the deuterium is in an equatorial position the observed effect is smaller than when it is an axial one.<sup>84</sup>

### $\beta$ -shifts

The effect over two bonds, a  $\beta$ -shift, is always smaller than the value of an  $\alpha$ -shift which is only over one bond and is usually less than 0.1 ppm. Under high resolution  $\beta$ -shifts are easily observed and the line-broadening caused by unresolved carbon-deuterium coupling is usually negligible since  $^2J(\text{C-H})$  and hence  $^2J(\text{C-D})$  is small. The  $\beta$ -shift for open-chain hydrocarbons is usually about +0.08 ppm while cyclic hydrocarbons vary with the size of the ring i.e. Cyclopropane < Cyclobutane ~ Cyclobutene > Cyclopentene ~ Cyclohexene ~ Cycloheptane.<sup>82</sup> A small difference between  $D_{\text{eq}}$  and  $D_{\text{ax}}$  is observed in cyclohexane. This difference can also be seen in cyclohexane- $D_{11}$  and cyclohexane- $D_{12}$ .<sup>84</sup>

$\gamma$  - shifts

The isotope effect over three bonds, a  $\gamma$ -shift, is small. On many occasions they are ignored since they are not resolved under experimental conditions. Mazurek<sup>77</sup> found 0.02 ppm per deuterium in long chain hydrocarbons and in cyclohexane an average value of 0.02 ppm is observed.<sup>82,83</sup>

C sp<sup>3</sup>D C sp<sup>2</sup> effects

Very few cases have been reported. Cyclobutene 1-D values for  $\beta$ -shifts are approximately +0.159 ppm and  $\gamma$ -shifts of about 0.053 ppm.

C sp<sup>2</sup>D C sp<sup>3</sup> effects

This effect is usually found in alkyl-substituted aromatic systems and also in carbonyl derivatives as well as carbonium ions.

 $\beta$ -shifts

Deuteriated toluenes show an additive  $\beta$ -effect of 0.034 ppm per deuterium. The isotope effect is much smaller than that observed for cyclobutene-3D which was +0.078 ppm. The two bond isotope effect of acetone was the first negative effect to be reported (-0.28 ppm) with the negative value being ascribed to hyperconjugation.<sup>85</sup> Negative values although smaller, have been reported for substituted camphors.<sup>86</sup> In acids or acid derivatives large  $\beta$ -shifts have been reported for formic acid derivatives but in these an

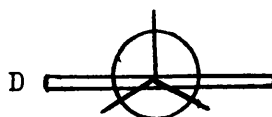
$\alpha$ -effect is also present. Small  $\beta$ -shifts are observed in  $D_6$ -ethyl acetate. A large number of carbonium ions have been investigated with both positive and negative  $\beta$ -effects being observed.<sup>87</sup>

### $\gamma$ -shifts

Unusual negative isotope effects over three bonds are observed in cyclobutene-3 $D$ <sup>87</sup> and in alkylbenzenes with deuterium at the  $\alpha$ -carbon.<sup>88,89</sup> The  $\gamma$  shift for toluene- $\alpha$ -D, ethylbenzene- $\alpha$ -D and isopropylbenzene- $\alpha$ -D increases from -0.2 ppm to +14.3 ppm. The two conformations (I) and (II) shown below suggest that (II) is probably more highly populated which means that hyperconjugation becomes less favourable and hence a positive value is observed.



(I)



(II)

### C sp<sup>2</sup>D C sp<sup>2</sup> Systems

This combination is seen frequently in olefins and aromatic systems which also includes heteroatoms.

### $\alpha$ -shifts

In olefins the  $\alpha$ -shifts usually fall close to 0.21 ppm, while in aromatic hydrocarbons they are slightly larger, being in the range +0.24 to +0.31 ppm.



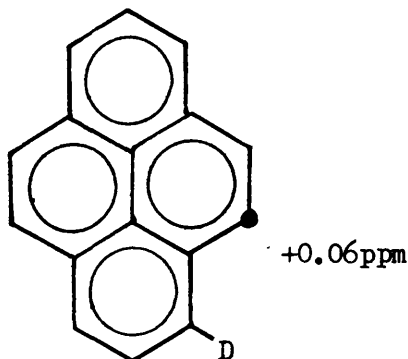
Substituent effects were first studied by Bell et al<sup>90</sup> who found that both  $\alpha$  and  $\beta$ -shifts are influenced by substituents.  $\alpha$ -Shift values of heteroaromatics are clearly controlled by the nature of the heteroatom and possibly also by the degree of aromaticity.

### $\beta$ -shifts

The effects observed in olefins and aromatics are of the same magnitude (approximately 0.1 ppm). In polycyclic aromatics,  $\beta$ -shifts have varied from +0.05 to +0.12 ppm.<sup>91</sup> In a compound containing two ortho carbons the larger shift value is associated with the larger  $\pi$ -bond or length of the C-C bond.

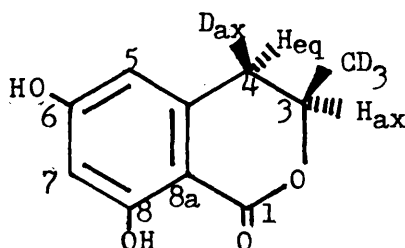
### $\gamma$ -shifts or more

$\gamma$ -Shifts are relatively small in benzene derivatives but are much larger and hence easier to observe in polycyclic aromatics in a peri position (129).



(129)

Spectroscopic analysis of 4[ $\alpha,\alpha,\alpha$ - $^2\text{H}_4$ ]-3,4-  
dihydro-6,8-dihydroxy-3-methylisocoumarin (107)



(107)

$^1\text{H}$  N.m.r.

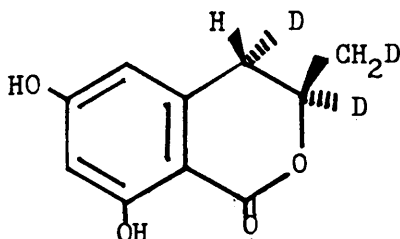
The 200 MHz  $^1\text{H}$  n.m.r. spectrum of (107) was analysed and the following peaks observed in the spectrum. A multiplet centred at  $\delta 6.33$  was found, this is due to the two aromatic protons present in the isocoumarin at C-5 and C-7. A singlet at  $\delta 4.72$  is due to the pseudo-axial proton at C-3. This singlet is slightly broadened due to some coupling with the deuterium atom attached to C-4. From the n.m.r. spectrum there appears also to be a small amount of deuterium present at C-3 which could be a result of deuterium exchange on the catalytic surface.

At  $\delta 2.96$ , a doublet can be seen with a small coupling constant of 2.5 Hz. There is also a very small peak at  $\delta 1.48$  which is due to a little  $\text{CH}_3$ , although the majority of hydrogens in the methyl group have been replaced by deuterium. From the information above it can be concluded that the large coupling constant of 12 Hz which was observed in the undeuteriated isocoumarin(90) between the pseudo-axial proton of C-4 and the pseudo-axial proton of C-3 has

all but disappeared. However, the small coupling constant still remains and must be due to the pseudo-equatorial-pseudo-axial splitting. Consequently it is the pseudo-axial proton at C-4 which must have been replaced by a deuterium as was hoped.

In order to conclusively confirm the structure of (107) a  $^{13}\text{C}$  n.m.r. spectrum was obtained. The position and number of lines observed in the spectrum were very similar to the  $^{13}\text{C}$  n.m.r. spectrum of the undeuteriated isocoumarin (90). However there were a few differences:- the line at 34.50 ppm, due to C-4 of the isocoumarin is seen as a triplet. This is due to the fact that there is coupling with the deuterium attached to it. Deuterium has a nuclear spin of 1 and so a  $^{13}\text{C}$  D will appear as a triplet of lines in the  $^{13}\text{C}$  n.m.r. This confirms that only one deuterium is attached to C-4. There is a negligible signal at 20.70 ppm, which must be due to the presence of a little undeuteriated-methyl group. However, in almost all of the molecules the  $-\text{CH}_3$  group has been replaced by a  $-\text{CD}_3$  grouping. From the evidence seen above it can be concluded that the structure indicated for the isocoumarin (107) is indeed the correct one.

Spectroscopic analysis of  $[3,4-^2\text{H}_2]$ -3,4-dihydro-6,8-dihydroxy-3-methyl isocoumarin (108)



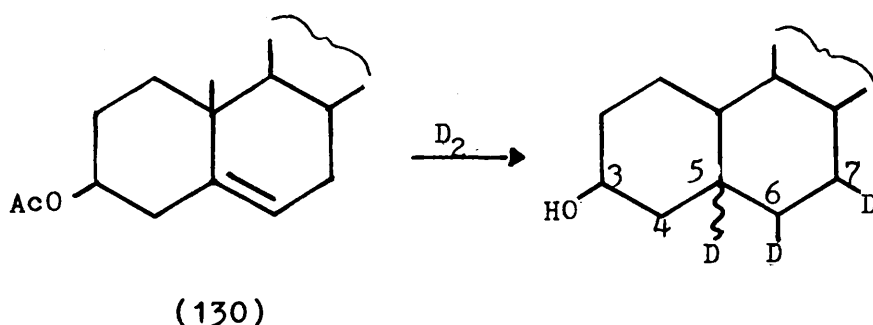
(108)

$^1\text{H}$  n.m.r.

Analysis of the  $^1\text{H}$  n.m.r. spectrum of (108) showed a singlet of intensity two to be present at  $\delta 6.28$ . This is due to the two aromatic protons attached to C-5 and C-7 of the isocoumarin. It can also be seen that the peak at  $\delta 4.67$  which was due to the proton at C-3 of the unlabelled isocoumarin has now been replaced by a deuterium. The proton at C-4 is seen as a broad singlet due to some C-D coupling. The small coupling constant which was present in the unlabelled isocoumarin has disappeared with only the large coupling constant remaining. Thus the deuterium is in the pseudo-equatorial position at C-4. From the  $^1\text{H}$  n.m.r. spectrum it also appeared that the terminal methyl group contained at least one deuterium which must have exchanged for a proton during the reaction. This was an unexpected result in that more than two deuteriums had been introduced into the molecule. Fukushima and Gallaher<sup>92</sup>

reported a similar occurrence in their study of the catalytic reduction of a variety of steroids with deuterium.

They found that sixteen per cent of the total isotope incorporated appeared at C-7, that is the carbon which is immediately adjacent to the double bond. It was also noteworthy that no isotope substitution was found at C-4.



An analogy can be drawn between the reduction of cholesterol (130) and the reduction of the isocoumarin (122) with deuterium gas. Fukushima proposed a mechanism for the deuteration of cholesterol which could be adapted for the deuteration of the isocoumarin.

A plausible mechanism is shown in scheme 27. This involves the initial formation of a complex of type I. Attack of a deuterium at either carbon 3 or 4 would give two different species of type II and IV respectively. Since carbon-4a is a tertiary carbon, species II cannot lose hydrogen to form a different complex to I. Species II can however be reduced further to give (123) which can be removed from the surface of the catalyst by de-adsorption. Species

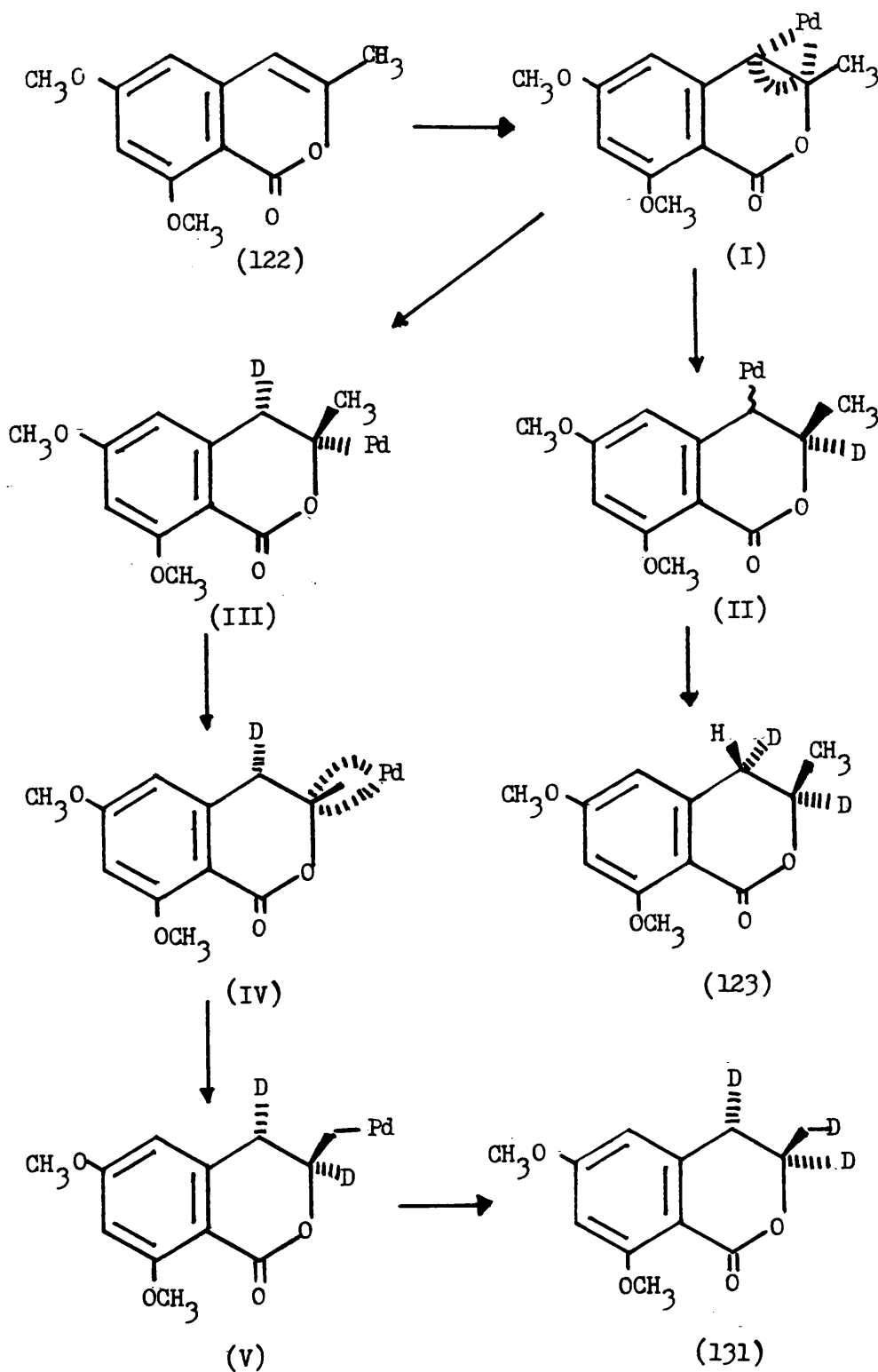
III, however may continue on to form the intermediate IV by dissociation of the hydrogen at carbon-9.

Reduction of IV would give V which could be further reduced to the trideuteriated isocoumarin (131).

### $^{13}\text{C}$ N.m.r.

In the  $^{13}\text{C}$  n.m.r. spectrum of the isocoumarin (108),  $\alpha$  and  $\beta$ -deuterium shift effects are observed. The peak at 101.86 ppm is due to C-5 of the isocoumarin and is seen to have a  $\beta$ -shift of 0.343 ppm. This is unusual in that it is quite large but is probably due to the cumulative effect of all the other deuteriums in the molecule. Carbon-3 of the isocoumarin is seen as a triplet at 76.25 ppm due to some  $^{13}\text{C}$ -D coupling. An  $\alpha$ -shift of 0.39 ppm is observed due to the presence of deuterium at C-3. A  $\beta$ -shift of 0.026 ppm is also seen due to the deuterium at C-4 and the methyl group deuterium. For C-4 an  $\alpha$ -shifted triplet is observed with the value of 0.34 ppm. A  $\beta$ -shift of 0.13 ppm is also seen. The terminal methyl group of the isocoumarin has already been shown by  $^1\text{H}$  n.m.r. to contain at least one deuterium. In the  $^{13}\text{C}$  n.m.r. it is seen to be  $\beta$ -shifted by 0.13 ppm due to the deuterium at C-3. There is also an  $\alpha$ -shifted triplet of 0.27 ppm which is due to the deuterium exchanging for a hydrogen which was discussed above. An  $\alpha$ - and  $\beta$ -shifted triplet can also be seen due to the CD-CH<sub>2</sub>D grouping. The  $^{13}\text{C}$  n.m.r. spectrum shows that

a very small amount of molecules contain two deuteriums in the methyl group. From the information obtained from the  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectra of (108) it can be concluded that the correct structure has been proposed for this compound.

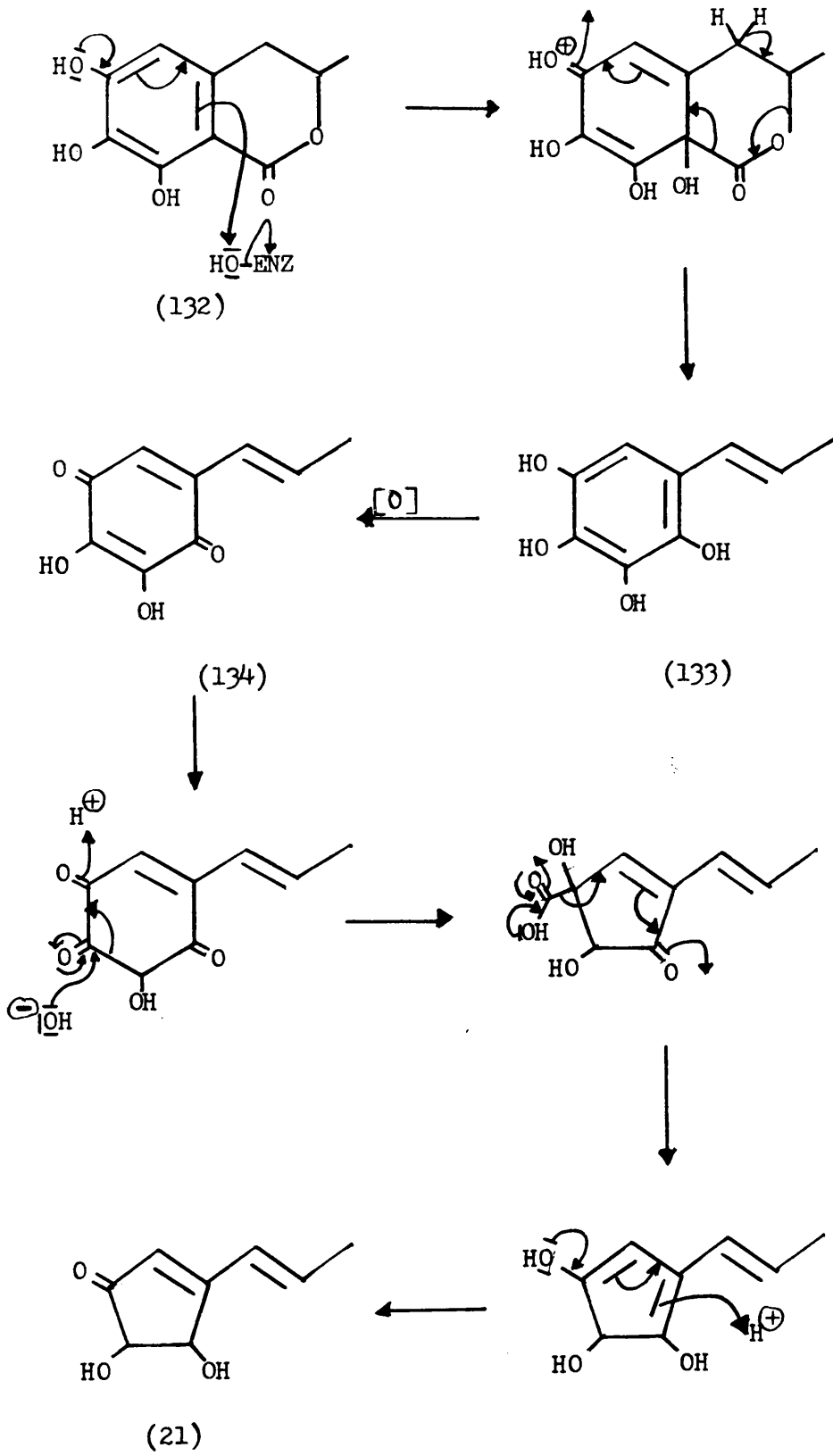


Chapter 3B.Synthesis of trihydroxyisocoumarins.

In the light of the discovery that the dihydroxy isocoumarin (90) was a biosynthetic intermediate in the biosynthesis of terrein, it was thought not to be unreasonable to postulate that the trihydroxyisocoumarin (132) may also be a metabolite on the pathway to terrein. As discussed in Chapter 2, Hill and co-workers have identified that C-7 of the dihydroxy isocoumarin is lost by way of a ring contraction in the course of the biosynthesis. It seems likely that this carbon must contain some degree of oxygenation if it is to be extruded from the six-membered ring as either carbon dioxide or formic acid. Thus it was proposed that the trihydroxyisocoumarin (132) could be a possible metabolite in the biosynthesis of terrein.

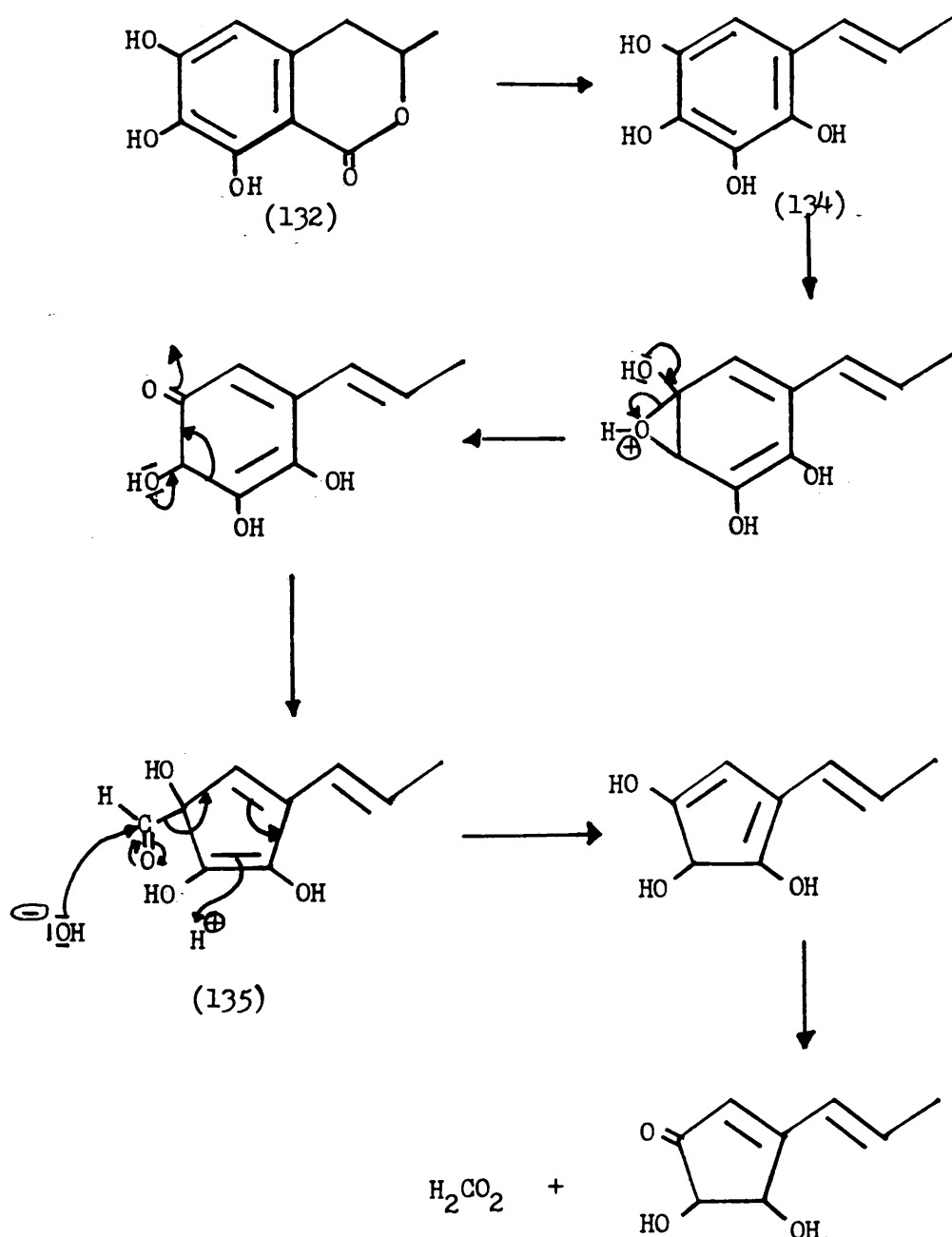
There are several possible biosynthetic pathways that would enable the formation of terrein from the trihydroxyisocoumarin. If the isocoumarin (132) is hydroxylated enzymically as was proposed for the dihydroxy-case (See Scheme 23), then a similar mechanism could be proposed for the trihydroxyisocoumarin leading to the tetrahydroxyphenylpropene intermediate (133). This could then be further oxidised to the quinone (134). Hydroxylation at C-7 followed by ring contraction, losing C-7 as carbon dioxide would lead to the required structure, terrein. (Scheme 28)





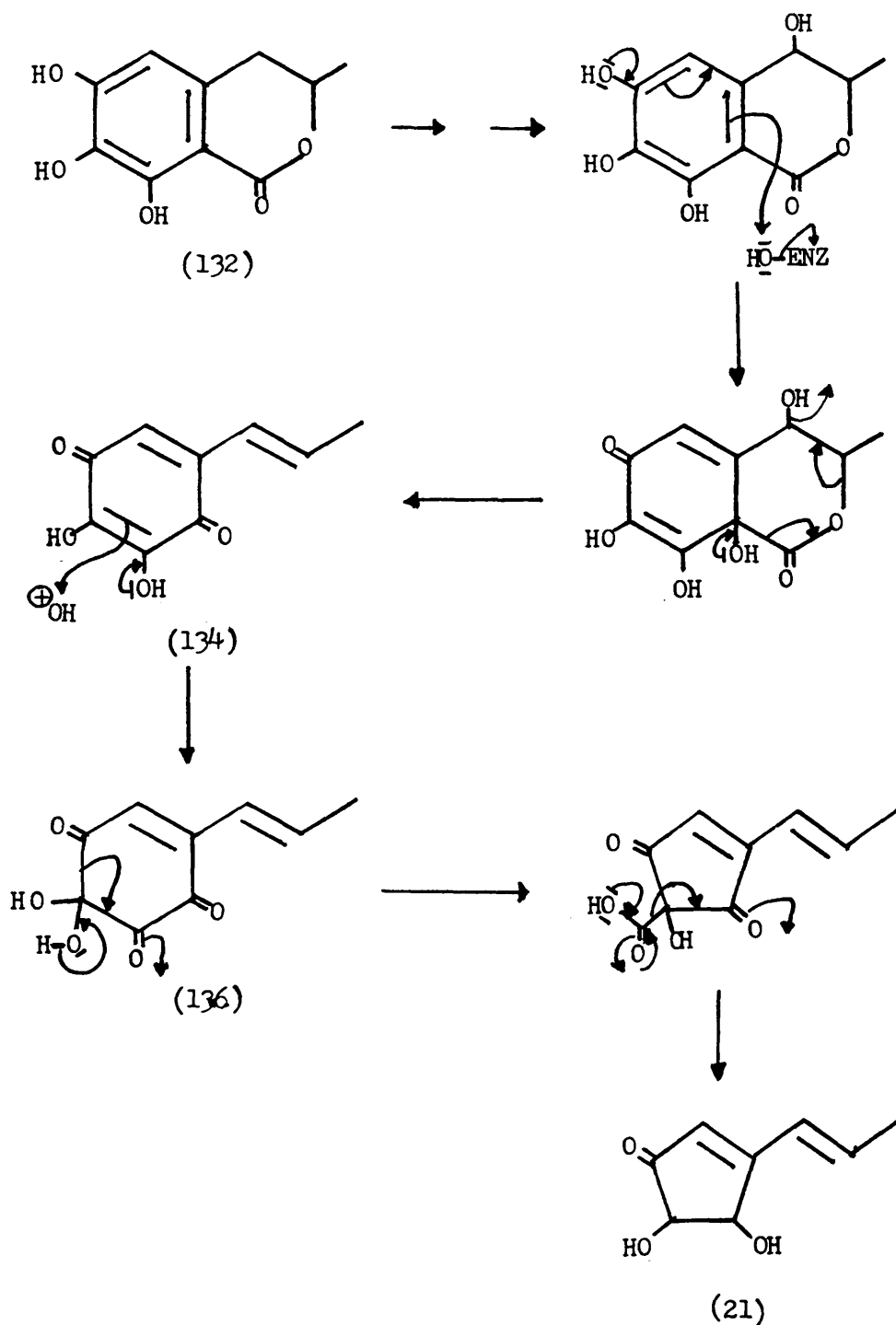
Scheme 28

Another possible biosynthetic pathway from the trihydroxyisocoumarin to terrein could be via epoxidation across the 6-7 bond of the tetrahydroxy-phenylpropene (134). Subsequent ring contraction with loss of C-7 would lead to an intermediate of type (135) which on decarboxylation would afford terrein. Scheme (29)



Scheme 29

However, it could also be the case that the trihydroxyisocoumarin undergoes hydroxylation at C-4 and C-8a to give the quinone intermediate (134) indicated in scheme 30. This quinone could then be hydroxylated at C-7 to give the unstable triketone (136) which will immediately undergo ring contraction with the loss of C-7 as carbon dioxide to give terrein.

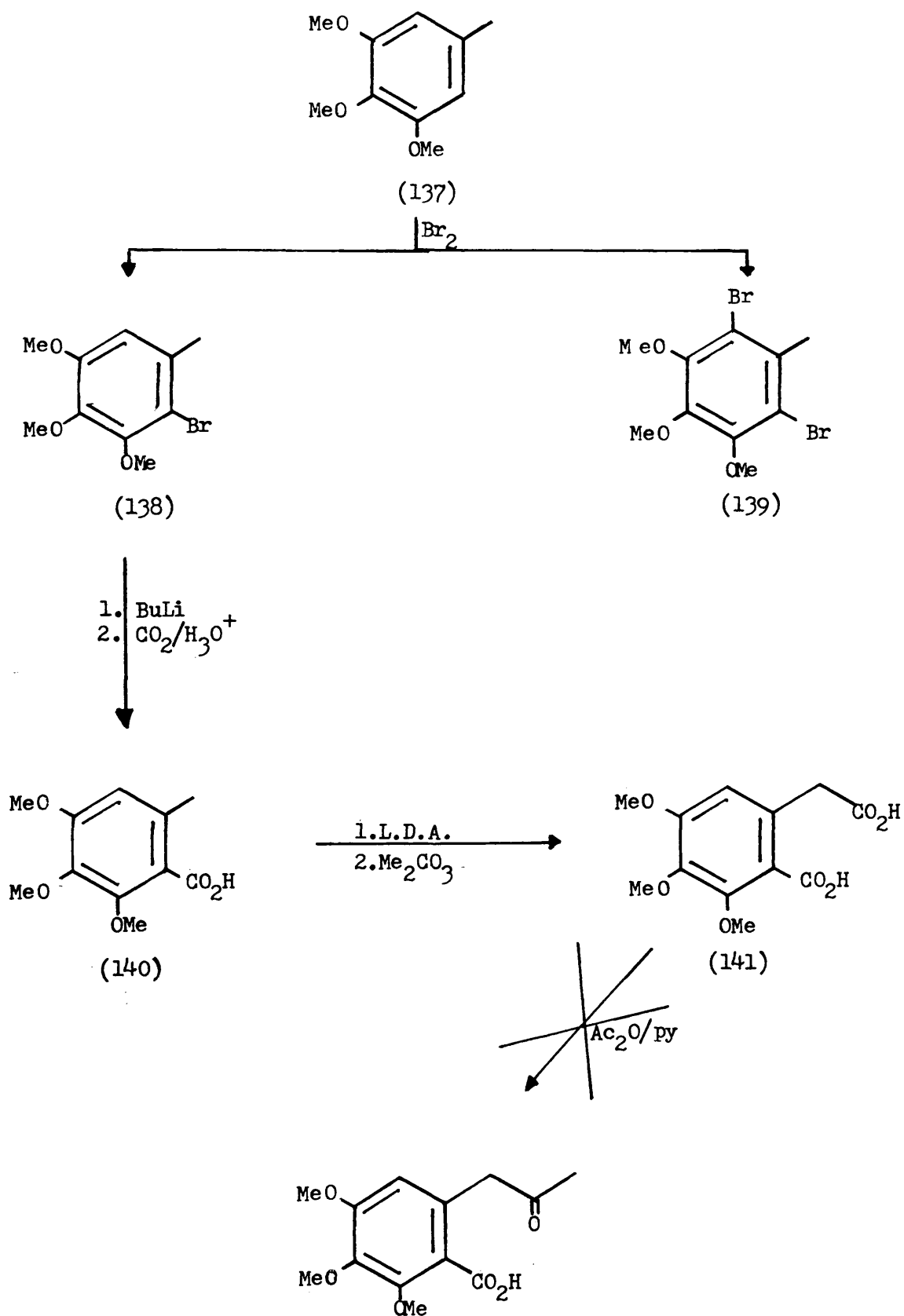


Scheme 30

The trihydroxyisocoumarin (132) was thought to be a possible intermediate on the biosynthetic pathway to terrein and the decision was made to set about synthesising it. On analysis the best method to approach a synthesis of the isocoumarin appeared to be via the route that was previously used to make the dihydroxyisocoumarin. (Scheme 31)

The initial starting point was from 3,4,5-trimethoxytoluene (137) which is commercially available from the Aldrich Chemical Company. This trimethoxy compound (137) was brominated to give a mixture of three compounds, starting material, 2-bromo-3,4,5-trimethoxytoluene (138) and 2,6-dibromo-3,4,5-trimethoxytoluene (139) in a ratio of 1:4:1 respectively. These were separated by column chromatography enabling the pure mono bromo compound (138) to be obtained in a 60% yield. Treatment of (138) with n-butyllithium produced the 2- lithio species which was quenched with carbon dioxide giving the benzoic acid (140). This can be converted into the corresponding homophthalic acid (141) by reacting the benzoic acid (140) with lithium diisopropylamide followed by dimethyl carbonate. Hydrolysis of the ester with acid furnished 3,4,5-trimethoxyhomophthalic acid (141) in an 83% yield.

3,4,5-Trimethoxyhomophthalic acid (141) was then treated with a mixture of acetic anhydride and pyridine in dry ether. The mixture was stirred for two hours at room temperature then heated at reflux with a 4 molar



Scheme 31

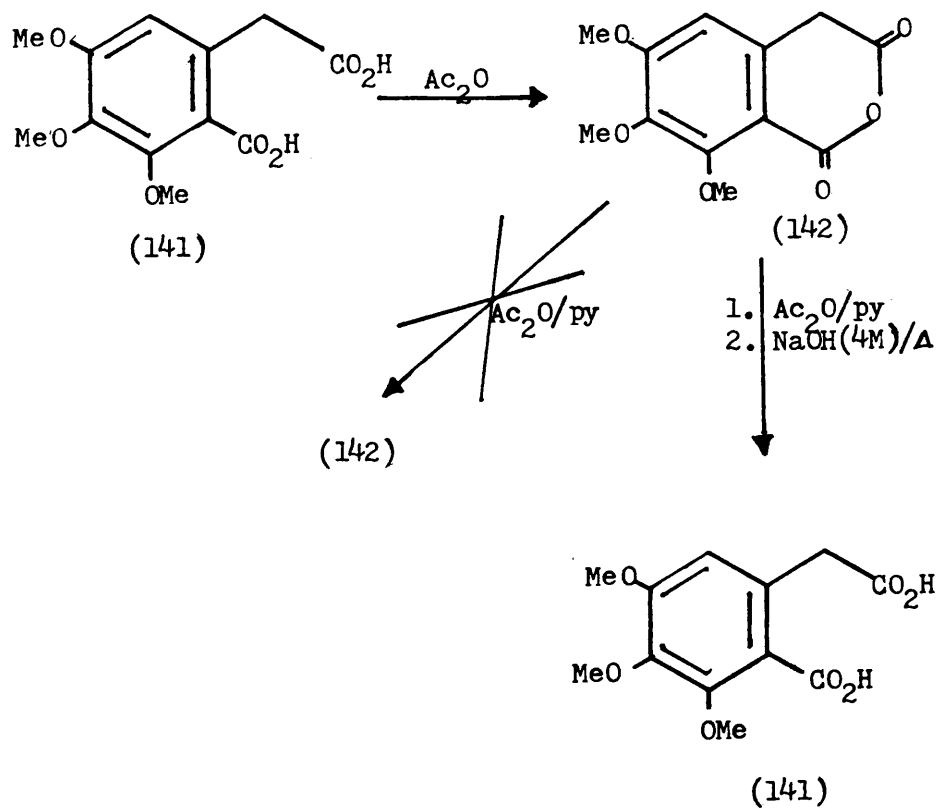
sodium hydroxide solution until all the solid material had dissolved, thus ensuring that hydrolysis had indeed taken place. Upon acidification and work up the only compound isolated from the reaction was found to be starting material. This reaction was repeated several times but on all occasions only 3,4,5-trimethoxyhomophthalic acid (141) was isolated. It was noted that the reaction appears to proceed in a visually different manner than it did for the dihydroxy-isocoumarin. On portionwise addition of the diacid to a stirred solution of acetic anhydride and pyridine the acid should dissolve after a couple of minutes after which a precipitate is normally formed. However, for the reaction with the trimethoxy acid, a much longer time spell is needed for the acid to dissolve. Thus, it is not clear how much of the acid and how much of the precipitate is present at any time or whether any precipitate is actually being formed at all.

To overcome this problem, it seemed sensible to make the homophthalic anhydride (142) which itself is thought to be an intermediate in the reaction.

3,4,5-Trimethoxyhomophthalic acid was stirred at room temperature with a solution of acetic anhydride for a  $\frac{1}{2}$  hour, which upon work-up yielded 3,4,5-trimethoxyhomophthalic anhydride (142). Reacting (142) with a mixture of acetic anhydride and pyridine followed by refluxing with a 4 molar sodium hydroxide solution furnished 3,4,5-trimethoxyhomophthalic acid (141).

Failure to reflux the mixture with a sodium hydroxide solution led to the recovery of the anhydride (142).

Scheme 32

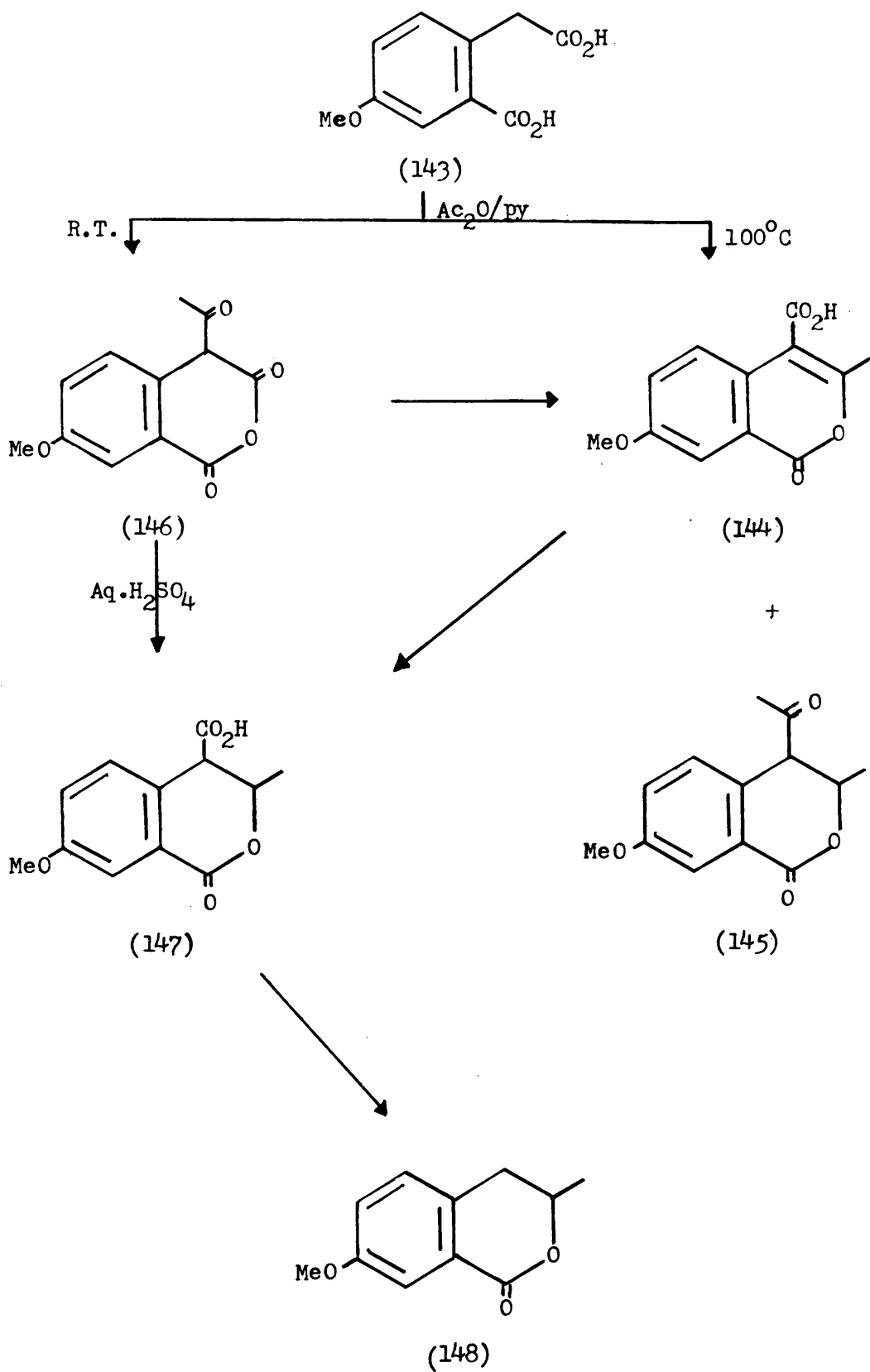


Scheme 32

A literature search revealed that there are numerous examples of pyridine-catalysed acetylations of homophthalic acids. The principal difference in the majority of these methods appeared to be in the ratio of acetic anhydride to pyridine that was used. Tirodkar and Usgaonkar<sup>93,94</sup> have reported the synthesis of 3-methyl-and 4-carboxy-3-methylisocoumarins, (148) and (147) respectively from homophthalic acids.

( Scheme 33 ) At 100°C the action of acetic anhydride on 4-methoxyhomophthalic acid (143) in the presence of pyridine furnished 4-carboxy-and 4-acetyl-7-methoxy-3-methylisocoumarins (144) and (145). However, if the reaction was carried out at room temperature the main compound isolated was the 4-acetylanhydride (146). This anhydride can be quantitatively converted into 4-carboxy-7-methoxy-3-methylisocoumarin (147) on treatment with aqueous sulphuric acid, getting partly decarboxylated to the isocoumarin (148) depending on the temperature of the reaction. At 0-10°C the major product isolated was the carboxy-derivative (147) but on carrying the reaction out by warming on a water bath for 45 minutes, the major product was found to be the decarboxylated isocoumarin (148).





Scheme 33

The anhydride (146) and the isocoumarins (147) and (148) were shown on treatment with aqueous sodium hydroxide to furnish a benzyl ketone derivative. The reaction naturally takes place by opening of the anhydride or lactonic ring to give a  $\beta$ -keto acid which can then be decarboxylated. These benzyl ketones were also shown to readily cyclodehydrate on keeping with 90% sulphuric acid to give a quantitative yield of 7-methoxy-3-methyl-isocoumarin (148).

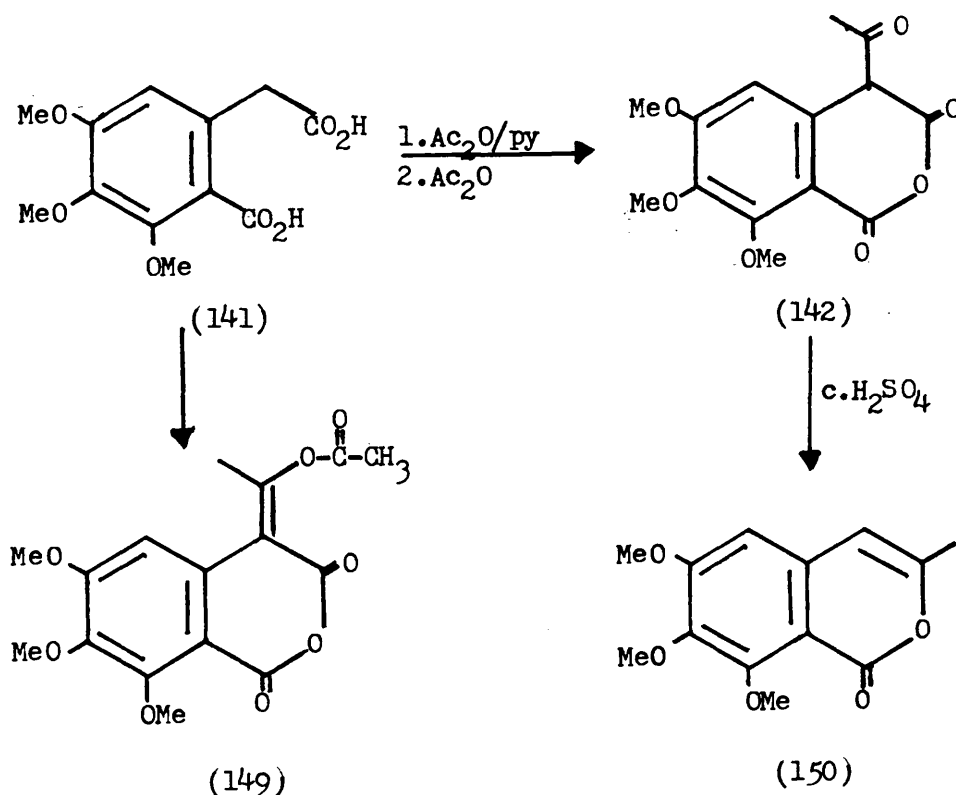
No reaction of acetic anhydride on mono and di-esters of 4-methoxyhomophthalic acids in the presence of pyridine have ever been seen to take place under these conditions, suggesting that the reaction takes place on the anhydride of 4-methoxyhomophthalic acid. This fact was further confirmed when the same products were obtained by the action of acetic anhydride and pyridine on the homophthalic anhydride as for the homophthalic acid. Acetylation of the methylene group in the anhydride is therefore probably the first stage of the reaction. The acetyl derivative then gets rearranged at higher temperatures, in the presence of pyridine.

The rearrangement also takes place in the presence of hydrogen ions (strong acid). It is important to note that a base like pyridine is essential for acetylation since no reaction was found to take place when the homophthalic acid was treated



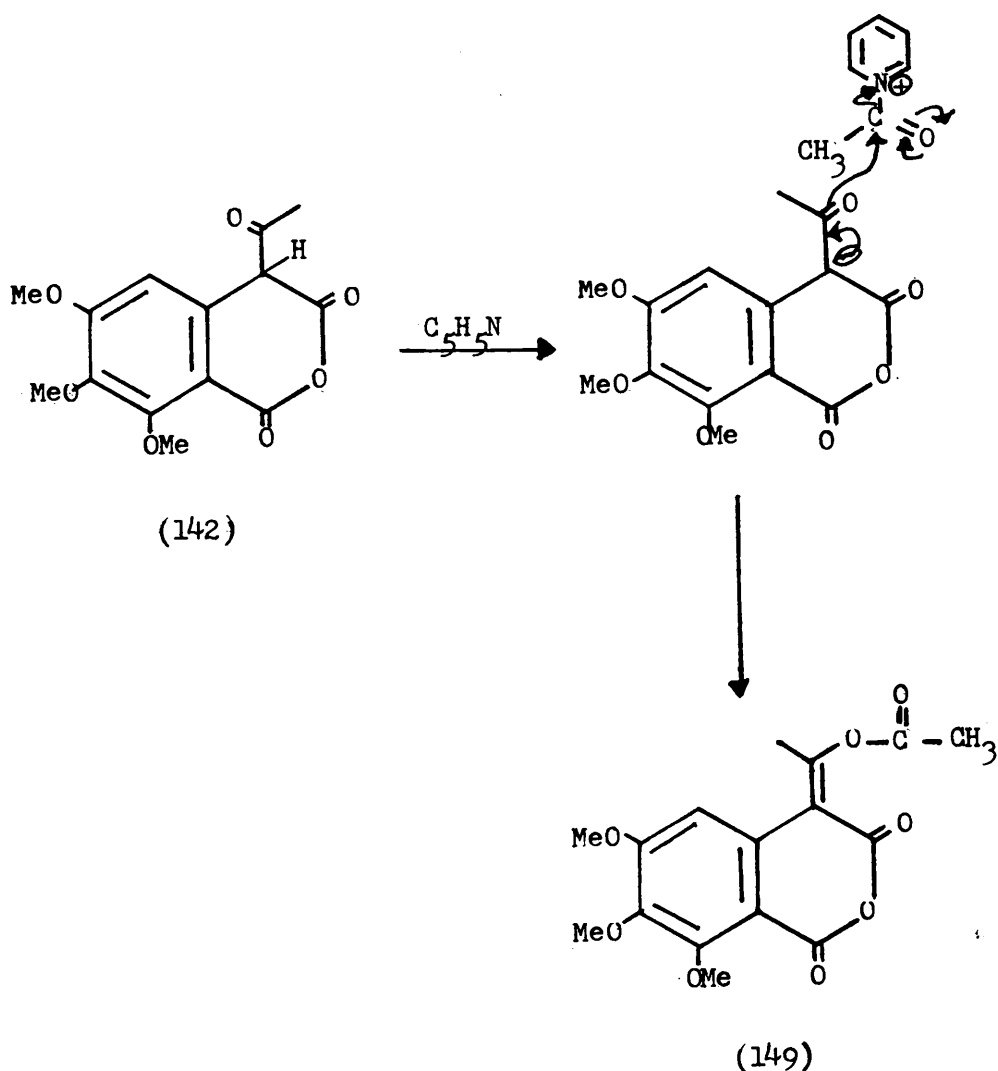
with acetic anhydride and concentrated sulphuric acid. The rearrangement may take place either via route A or B suggested in Scheme 34.

Thus it was decided to attempt making 5,6,7-trimethoxy-4-acetyl anhydride and consequently the required isocoumarin (150) by the method of Tirodkar and Usgaonkar.<sup>94</sup> 3,4,5-Trimethoxyhomophthalic acid was added in small portions to a mixture of acetic anhydride using the quantitative ratios indicated by the report. More acetic anhydride was added after 15 minutes and stirring continued for a further 1½ hours. On work-up the product isolated was analysed spectroscopically. Evidence gathered from the spectroscopic data suggested that the compound isolated had the structure (149) and was not the expected anhydride (142). (Scheme 35)



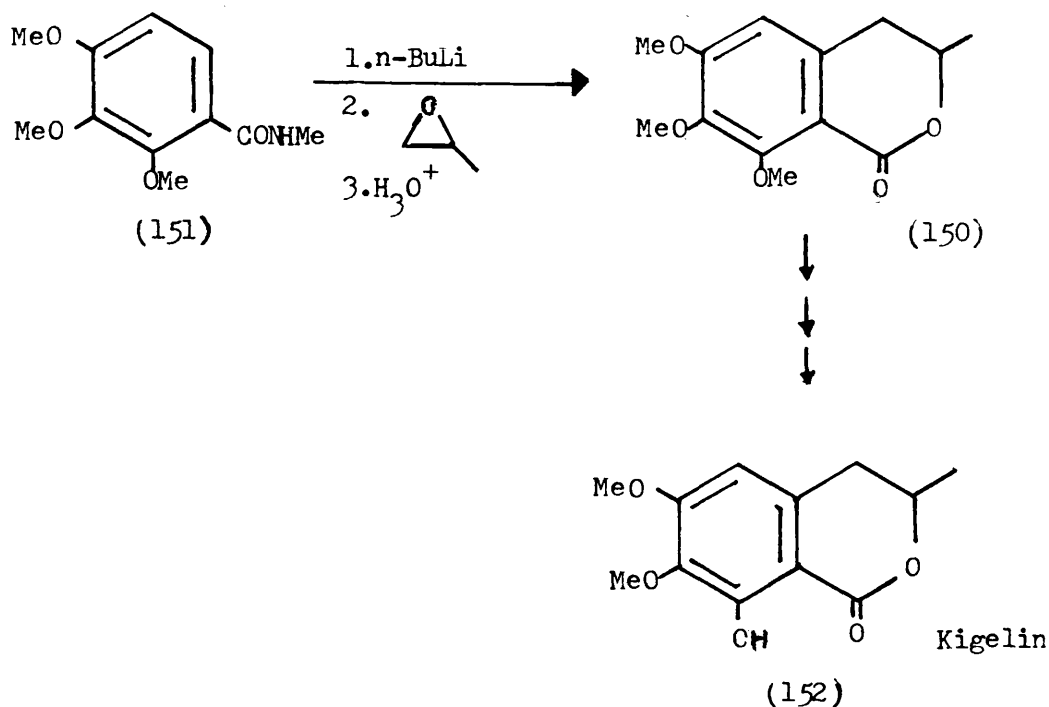
Scheme 35

Further attempts at carrying out the acetylation reaction by this method always resulted in the production of the unexpected product (149). This anhydride may be the result of further attack of (142) on another molecule of the acetylated pyridinium carbocation resulting in the production of the unusual anhydride (149). If this is the case then the normal isocoumarin (150) which was expected would be prevented from forming.



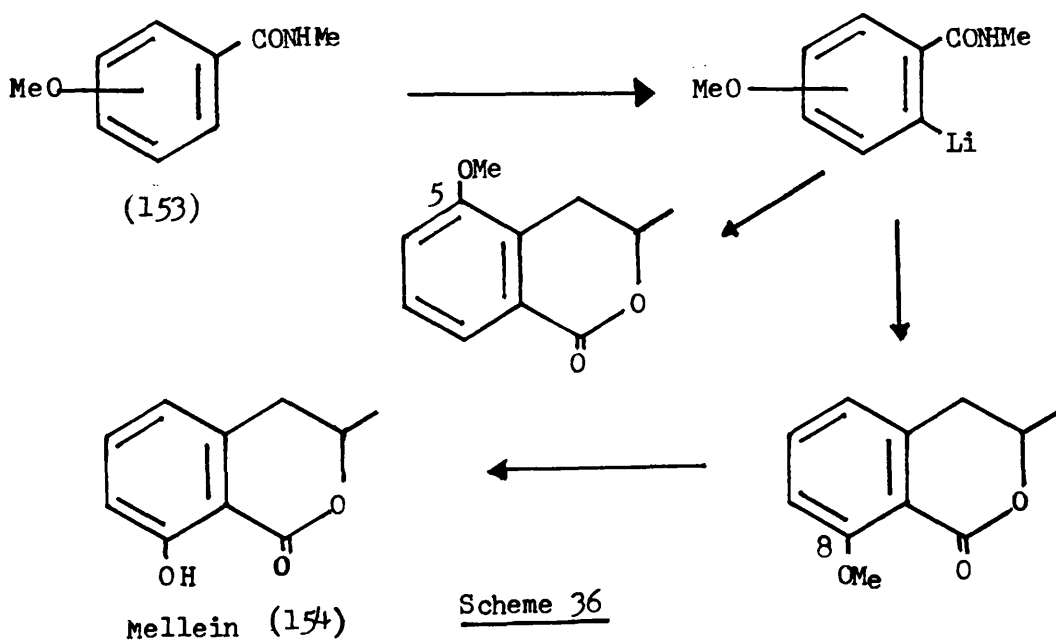
In the light of the failure of the acetylation reaction, it was decided to abandon this approach and find a different synthetic route from the homophthalic acid to the isocoumarin. On exploring the literature further it was noted that although there were many synthetic routes available to trihydroxyisocoumarins, only a few gave the required substitution pattern.<sup>96,97</sup>

In 1977 Bhide and Gupta<sup>98</sup> synthesised the phenolic dihydroisocoumarin kigelin (151). While doing so they reported that on route to Kigelin they had synthesised 3-methyl-5,6,7-trimethoxydihydroisocoumarin (150). This compound contained the correct oxygenation pattern which was required and it could easily be demethylated to the required trihydroxydihydroisocoumarin (132). Bhide and Gupta found that 3,4,5-trimethoxy-N-methylbenzamide (151) could be lithiated with an excess of n-butyllithium under a nitrogen atmosphere. The red metalation mixture formed was then condensed with propylene oxide. Alkaline hydrolysis afforded 3-methyl-5,6,7-trimethoxy-3,4-dihydroisocoumarin (150).



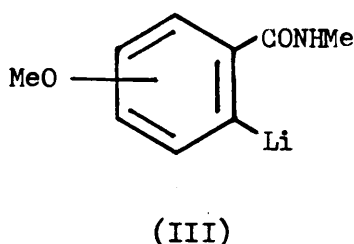
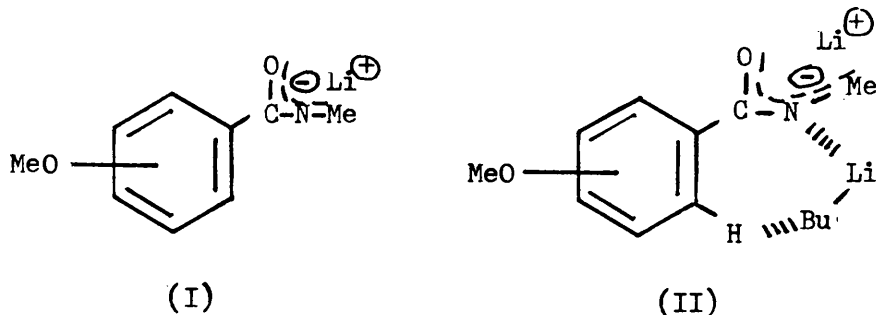
### Lithiation reactions<sup>99,100</sup>

Ring metalation ortho to an amide had previously been reported by Hauser<sup>101</sup> et al, who also extended it into a synthesis of isocoumarins. The simplicity of the reaction prompted Ehde and Narasimhan<sup>99</sup> to further develop it into a synthesis of 5- and 8-methoxy isocoumarins, (Scheme 36), and the naturally occurring 8-hydroxydihydroisocoumarin, mellein (154). Ehde and Gupta have now used it in the synthesis of 3,4,5-trimethoxyisocoumarins.



The starting materials for the synthesis of methoxy-isocoumarins are the corresponding methoxy-N-methylbenzamides (153). These compounds contain two functional groupings, the amide and the methoxy group, both of which are capable of controlling the position of entry of the lithium atom in the metalation reaction. The isocoumarin synthesis will succeed only if lithiation occurs ortho to the amide. The lithiation of methoxy-N-methylbenzamides was carried out with n-butyllithium in refluxing THF. The position of lithiation can be determined by treating the lithio-derivative with an electrophilic reagent like benzophenone and then looking at the nature of the benzohydrols formed. From the results obtained it was shown that lithiation of methoxy-N-methylbenzamides was occurring exclusively ortho to the amide group. This was presumably due to the fact that butyllithium is complexing better to the amide function (after replacement of the acidic hydrogen on nitrogen by lithium) than with the methoxy group. Hauser<sup>101</sup> had previously thought that N-methylbenzamide would undergo an initial N-metalation with n-butyllithium to form a monolithioamide (I). However if treated with excess of n-butyllithium then the dilithioamide transition state (II) would be formed since the carbonyl group in (I) is deactivated towards an addition type reaction. This intermediate (II) can then go on to form the ortho-lithiated intermediate (III).



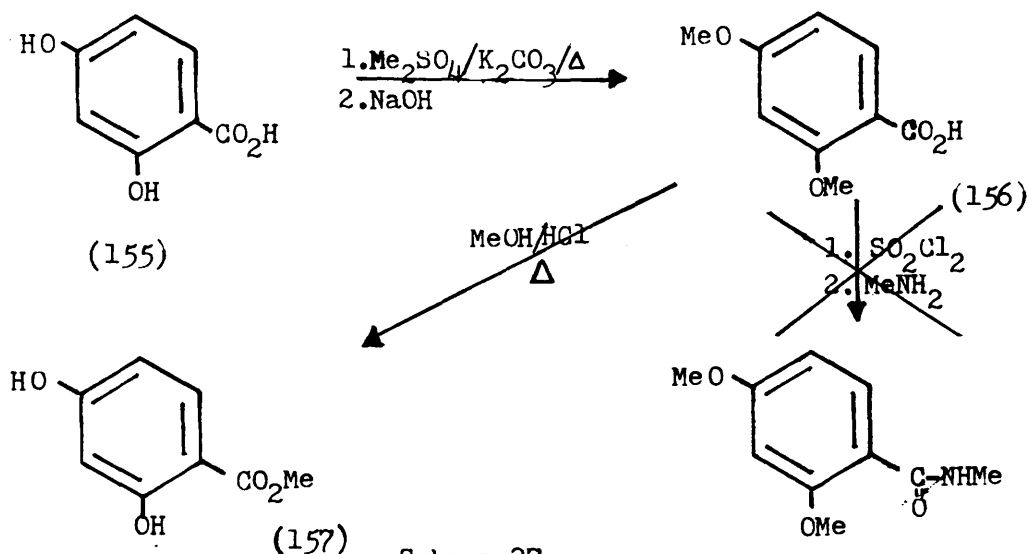


In the lithiation of m-methoxy-N-methylbenzamide an exceptionally good yield is normally obtained. This suggests that complex (II) is losing hydrogen ortho to the butyl group of the reagent as a proton. In this complex, the hydrogen which is ortho to the amide and the methoxyl group would be more acidic than the one which is ortho to the amide but para to the methoxyl group. The former is thus more reactive in a lithiation reaction. In these reactions it is noted that lithiation is in effect occurring at positions which are less reactive in acid-catalysed electrophilic substitution reactions.

The success of Bhide and Gupta in their synthesis of 3,4,5-trimethoxyisocoumarins by this lithiation method prompted an attempt at synthesising the required starting material for this reaction, 2,3,4-trimethoxy-N-methylbenzamide (151). This could then be reacted with excess n-butyllithium and the lithioamide formed condensed with propylene oxide.

The trimethoxy isocoumarin obtained could then be demethylated with boron tribromide affording the target compound (132).

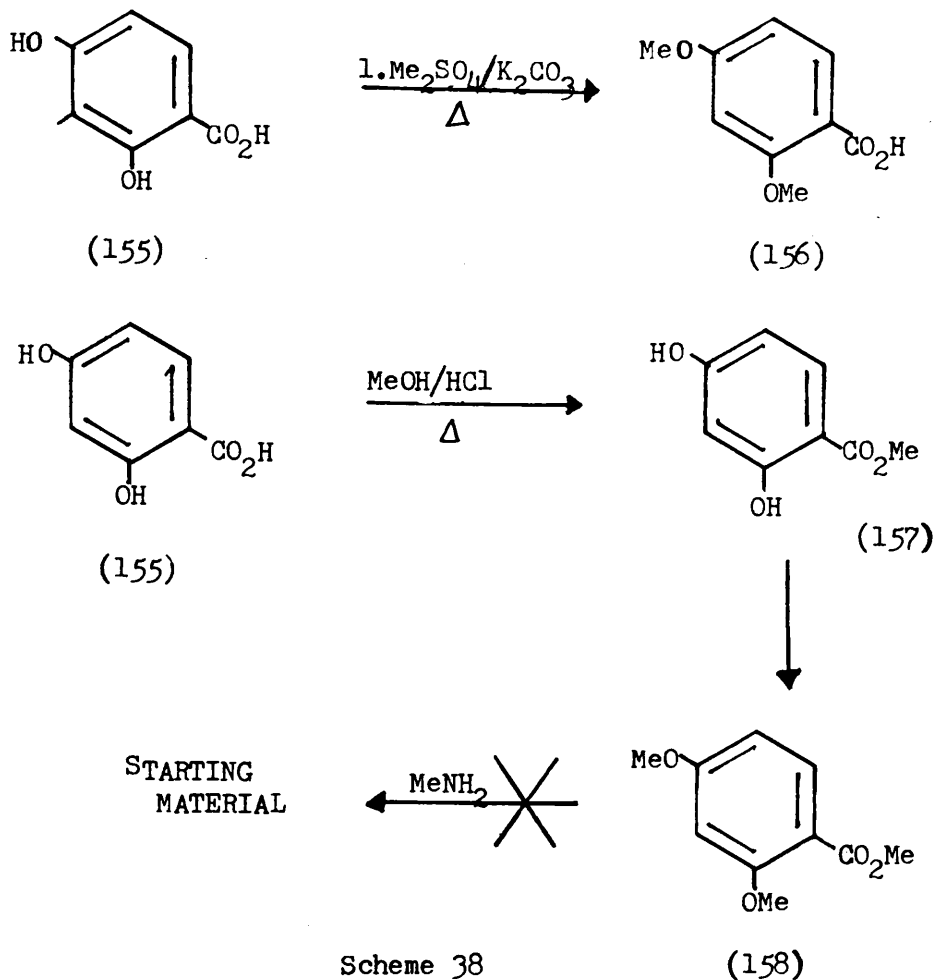
The best route to the required N-methylbenzamide (151) was thought to be via the acid chloride. It was first attempted on the dihydroxy acid (155). This was methylated with dimethylsulphate to give the protected benzoic acid (156). (156) was treated with an excess of thionylchloride and heated at reflux for 3 hours. The acid chloride was not isolated at this stage but immediately treated with a 25% methylamine solution in water at 0°C. Work-up did not furnish any of the required amide. It was then thought that it may be more advantageous to make the acid chloride via the methyl ester of (156) since a methoxyl would be a better leaving group than a hydroxyl. A solution of 2,4-dimethoxybenzoic acid in dry methanol was saturated with hydrogen chloride and the mixture heated at reflux for 4 hours. Methyl esterification was successfully found to have taken place, however in the course of the reaction the two methyl ether protecting groups had been found to be removed,affording methyl 2,4-dihydroxybenzoate (157).(Scheme 37)



Scheme 37

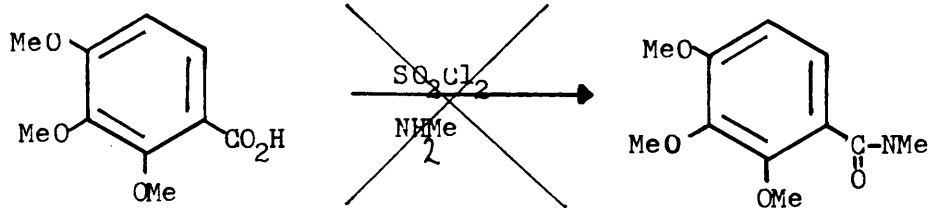
In a second attempt at making methyl 2,4-dimethoxybenzoate (158), 2,4-dihydroxybenzoic acid (155) was treated with dimethylsulphate and potassium carbonate in dry acetone, then refluxed for 6 hours. In the work-up the reaction was not refluxed with sodium hydroxide to avoid hydrolysis of the methyl ester. However work-up revealed that hydrolysis of the ester had indeed still taken place giving 2,4-dimethoxybenzoic acid (156) as the main product.

Treatment of the esterified acid, methyl 2,4-dihydroxybenzoate (157) with dimethylsulphate, again avoiding refluxing with base, work up revealed that the required dimethoxy ester had been produced. The route which was eventually used to acquire (158) involved esterification of 2,4-dihydroxybenzoic acid (155) with methanol and hydrogen chloride followed by methylation with dimethylsulphate. Treatment of methyl 2,4-dimethoxybenzoate (158) with methylamine did not furnish any of the required benzamide (Scheme 38).

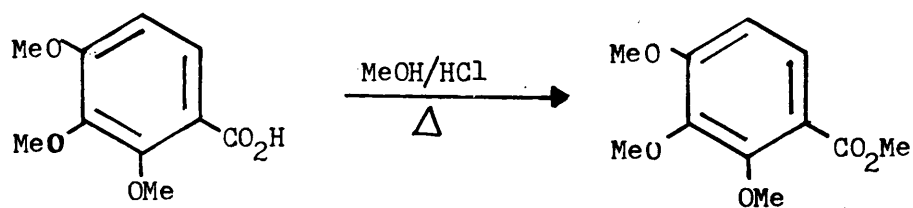


Although a lot of problems had been encountered on the route to the dimethoxy methyl ester it was decided to go ahead and attempt to make the trimethoxy ester.

2,3,4-Trimethoxybenzoic acid (159) which is commercially available, was heated at reflux with excess thionyl chloride. After 3 hours any excess thionyl chloride was distilled off and a 33% methylamine solution in water was added to the mixture. Analysis of the product showed only starting material to be present. Methyl 2,3,4-trimethoxybenzoate (160) was made successfully by saturating 2,3,4-trimethoxybenzoic acid with dry hydrogen chloride in a solution of

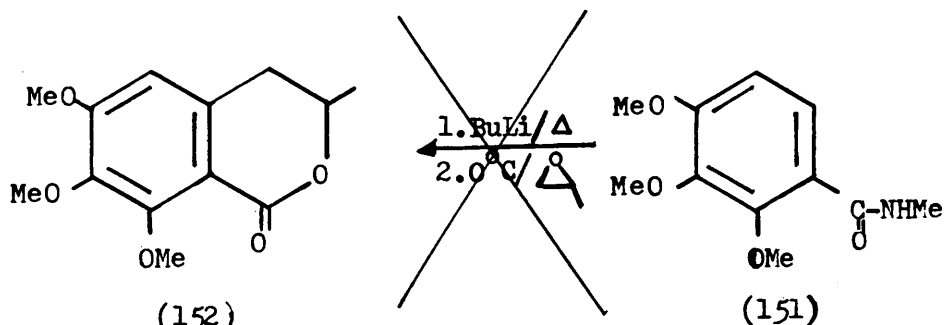


(159)



(159)

(160)

MeNH<sub>2</sub>

(152)

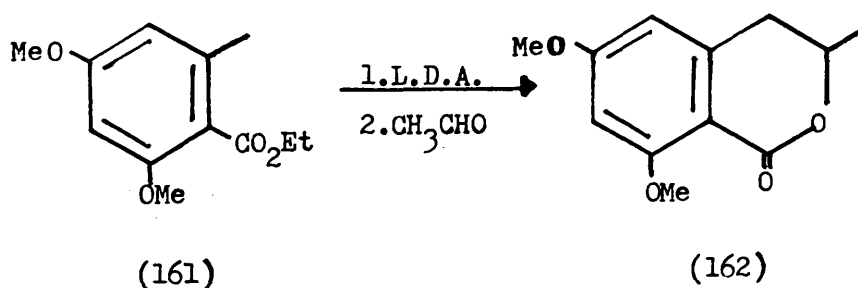
(151)

Scheme 39

methanol. The ester (160) was then treated with a 33% methylamine solution. Work-up afforded the required N-methylbenzamide (151) as seen in scheme 39.

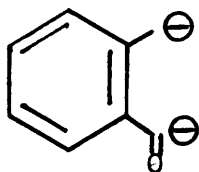
This benzamide (151) could now be subjected to the method previously used by Bhide for lithiating (151) with excess n-butyllithium. On addition of n-butyllithium a red metalation colour should be produced however no such colour was observed. However the mixture was still refluxed for 40 minutes and allowed to cool. Propylene oxide was then added at 0°C. Work-up yielded only starting material. The reaction was repeated several times on N-methyl-2,3,4-trimethoxybenzamide but on all occasions a red metalation colour was never observed indicating that the lithioamide had probably not been formed. This approach to the trimethoxyisocoumarin (152) was then abandoned. (Scheme 39)

A new approach to trihydroxyisocoumarin was yet again needed. In 1984 Staunton<sup>102,103</sup> et al reported that the o-toluate anion prepared from (161) could be condensed with acetaldehyde to give the isocoumarin (162).

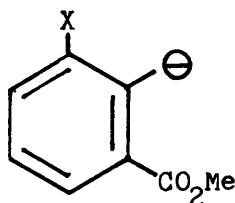


### Toluate anion chemistry

The synthon (161) is of great importance in the synthesis of linear polycyclic aromatic systems found in many antibiotics. It is for this reason that the toluate anion has received its fair share of attention, being an attractive starting point for the building up of this type of skeleton. The synthon (161) is effectively present in the toluate anions (162) and (163). Hauser and Rhee<sup>104</sup> reported that the toluate anion (161) dimerizes very rapidly and cannot be used synthetically. One method employed to stabilise o-toluate anions uses an electron-withdrawing substituent on the methyl group. Thus sulphides, sulfoxides and sulphones as well as methoxycarbonyl and cyano groups have all been used successfully. The unsubstituted phthalide anion (164) also appears more stable and has been used on several occasions. The use of electron-withdrawing groups to stabilize the anion and also act as a leaving group is also quite common.

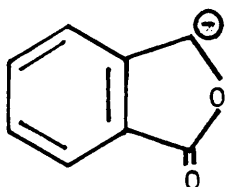


(161)

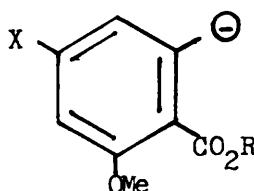


(162) X=H

(163) X=OMe



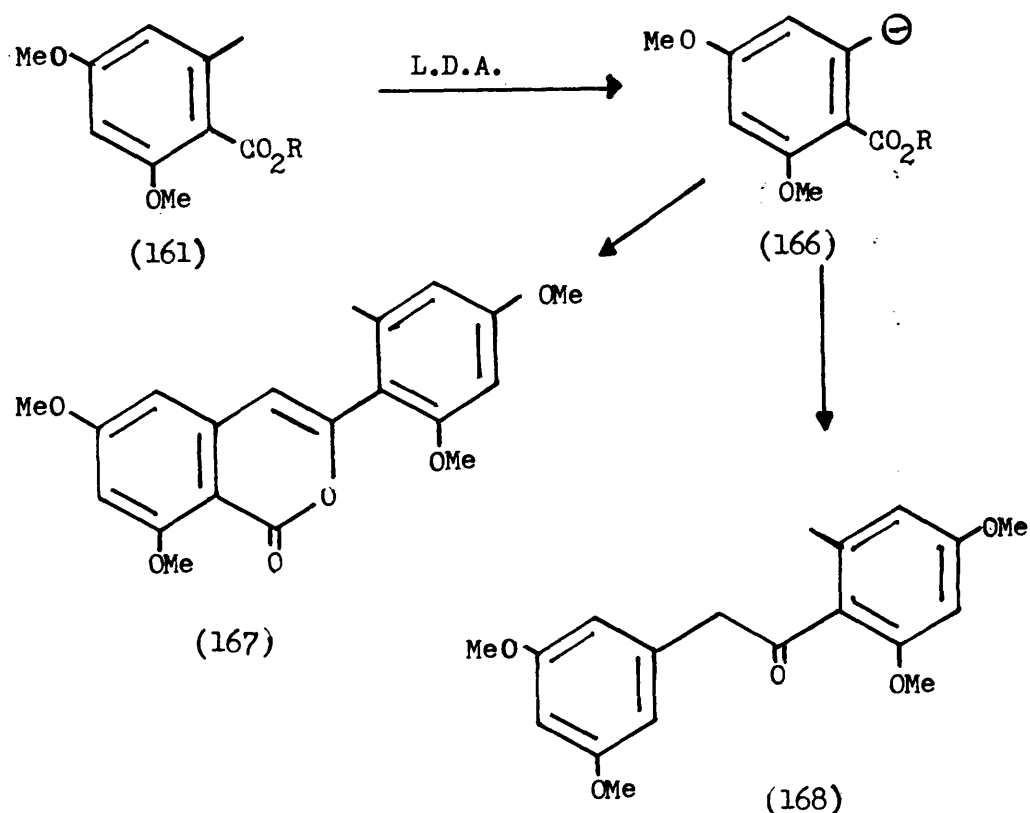
(164)



(165) X=H

(166) X=OMe

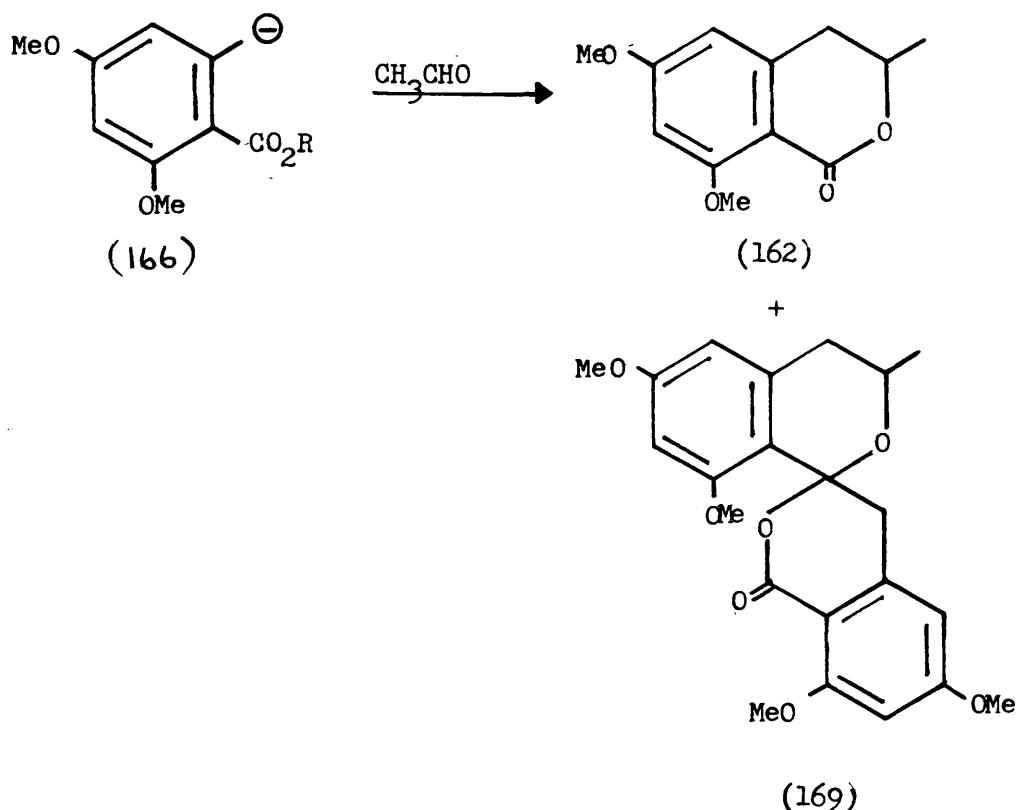
The o-toluate anions (165) and (166) which contain a methoxy substituent in the other position ortho to the carbonyl group are sufficiently stable at low temperatures to be synthetically useful without the need for any stabilising groups.<sup>105-107</sup> The extra stability of these anions over (162) and (163) is probably due to the second ortho substituent which hinders the carbonyl from nucleophilic attack. Staunton observed that on reaction of (161) with the <sup>non</sup>nucleophilic base lithium diisopropylamide, rapid deprotonation occurred at  $-78^{\circ}\text{C}$  to give the anion (166). This anion is stable for a few hours if kept at  $-78^{\circ}\text{C}$  but after longer periods or at higher temperatures decomposition will occur to give products such as (167) and (168) produced by self-condensation. (Scheme 40)



Scheme 40



Staunton found that acetylation of the anion (166) with aldehydes is accompanied by lactonisation of the intermediate. Thus, reaction of (166) with acetaldehyde gave the dihydroisocoumarin (162) and a major by-product (167) which arose by further attack of (166) on the product.



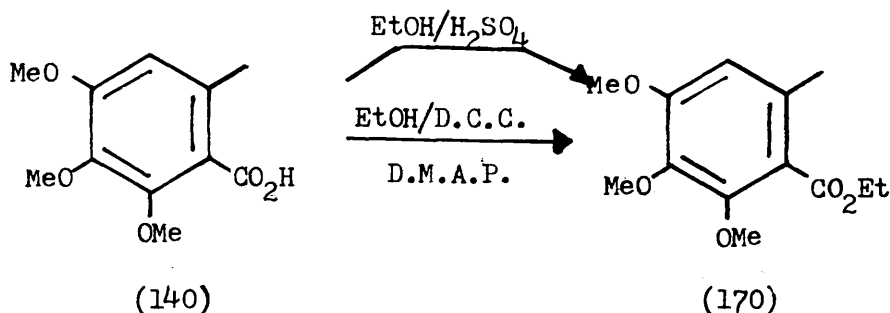
The increase in reactivity of the carbonyl group of the dihydroisocoumarin compared with the starting ester is probably because the C=O bond in the former can lie in the plane of the aromatic ring and so it is not hindered towards attack perpendicular to the plane. However in the starting ester the carbonyl is forced out of the plane by the bulky ortho substituents and consequently these substituents will hinder attack at

both faces. This side reaction can be eliminated by inverse addition of the anion to an excess of the aldehyde at  $-78^{\circ}\text{C}$ .

An attempt was made to synthesise 3,4,5-trimethoxy-isocoumarin by Staunton's route. One approach was via ethyl 3,4,5-trimethoxy-6-methylbenzoate (170).

2,3,4-Trimethoxy-6-methylbenzoic acid (140) was esterified with a mixture of ethanol and a few drops of sulphuric acid. Unfortunately only a small amount of the required ester (170) was obtained. The reaction was repeated using a longer reflux time but had no effect on the yield of product.

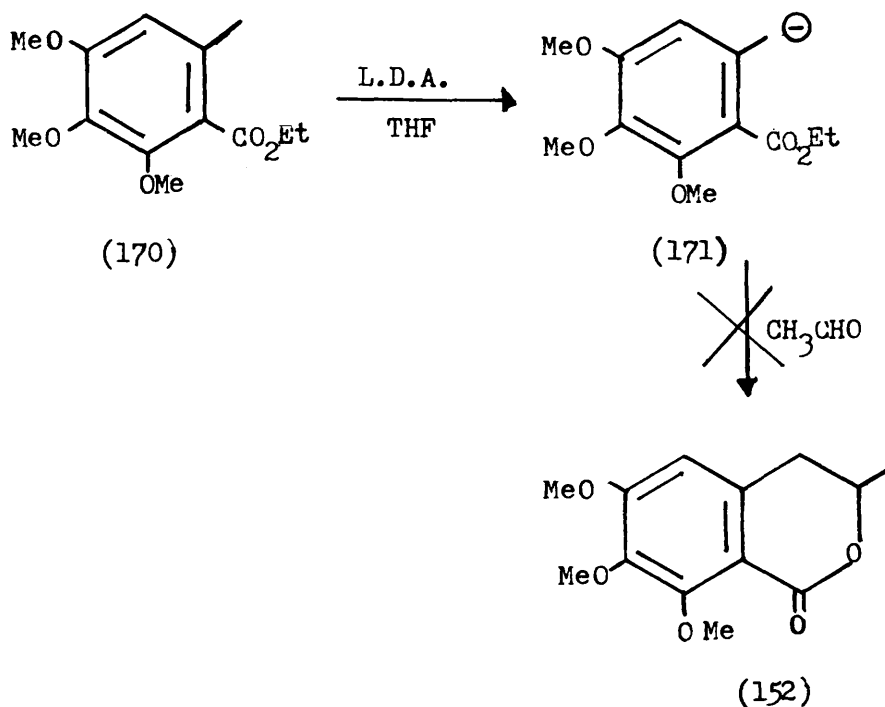
After several futile attempts at increasing the yield of the ethyl ester a new method was attempted. It was decided to try and convert the acid (140) into the ethyl ester (170) with the aid of dicyclohexylcarbodiimide (D.C.C.). Neises and Steiglich<sup>108</sup> have reported that addition of 3-10% of 4-dimethylaminopyridine (D.M.A.P.) accelerates the D.C.C. activated esterification of carboxylic acids to such an extent that the formation of any side products is suppressed even in sterically demanding esters. The D.C.C. method has not generally been adopted for the preparation of carboxylates chiefly because of its variable yields. The tendency for the formation of the undesirable N-acyl ureas is also a major drawback.



2,3,4-Trimethoxy-6-methylbenzoic acid (140) was converted efficiently into its ethyl ester using the D.C.C. method above.

With the ethyl ester (170) now at our disposal, it was possible to attempt a preparation of the required isocoumarin via Staunton's route.

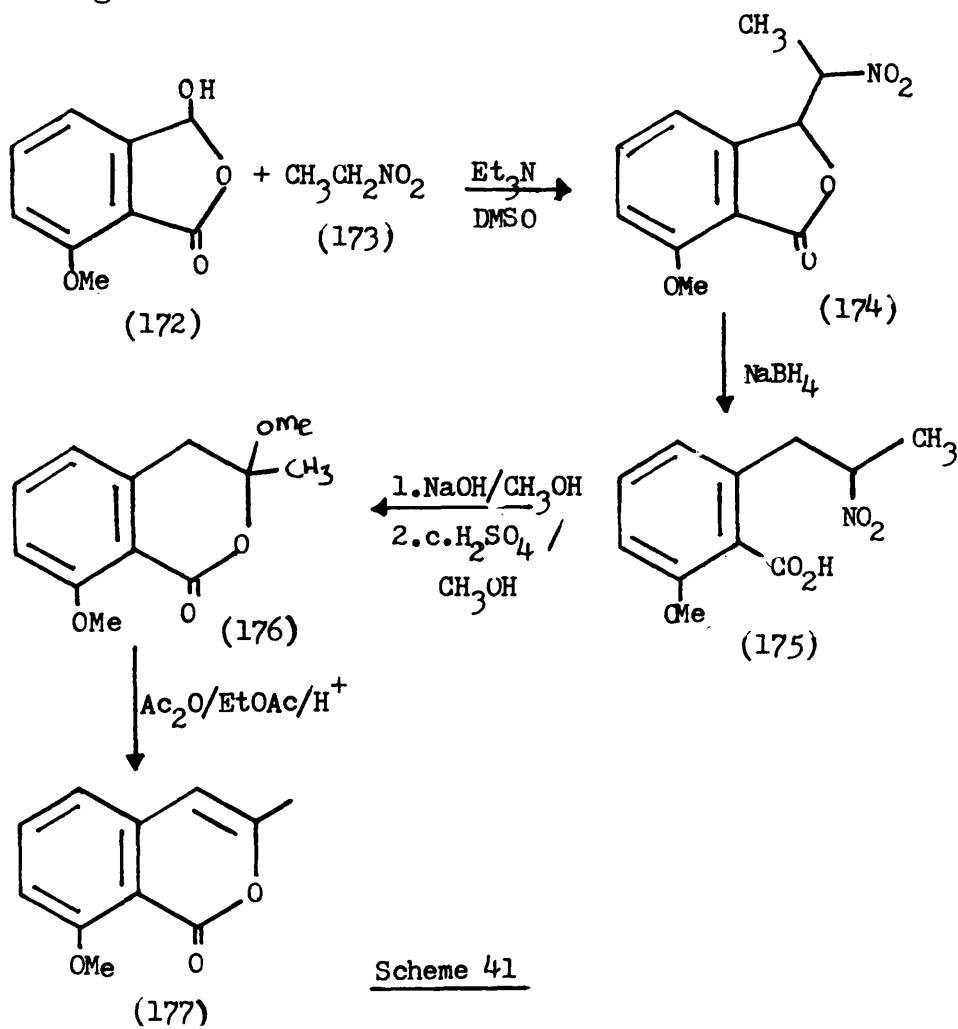
A solution of the anion 2-ethoxycarbonyl-2,3,4-trimethoxybenzyl lithium (171) was prepared by reacting diisopropylamine with n-butyllithium in THF followed by cooling to  $-78^{\circ}\text{C}$  and the addition of ethyl 2,3,4-trimethoxy-6-methylbenzoate (170). An orange-red colour was immediately observed indicating the formation of the anion. A solution of this anion was then added gradually to acetaldehyde. The colour of the anion solution was rapidly discharged after the addition of each portion. Work-up did not furnish any of the required trimethoxyisocoumarin (152).



A new synthetic route to 3-alkylbenzopyran-1-ones (177) has recently been developed by Hauser and Baghdanov.<sup>109</sup> The sequence starting from phthalaldehydic acids (172) and nitroalkanes (173) allows straightforward variation of both the 3-substituent and the pattern of functionalisation on the aromatic ring. The reaction conditions are relatively mild and the use of anhydrous conditions are unnecessary. In contrast to other syntheses, good yields of the 3-alkyl substituted isobenzofuranones are obtained.

The synthetic strategy was centred around the fact that nitroalkyl-substituted isobenzofuranones (174) produced from the Henry condensation of nitroalkanes with phthalaldehydic acids could be converted

to the 2-nitroalkyl acids (175) through reductive elimination. Subsequent transformation of the nitromethylene in (175) to a carbonyl group would give the keto-acid (176), which on intramolecular cyclisation and dehydration would yield the benzopyran-1-one (177). (Scheme 41) There are several literature reports describing the condensation of phthalaldehydic acids with nitroalkanes.<sup>110-112</sup> Hauser<sup>109</sup> found that the best conditions for Henry condensation of phthalaldehydic acids with nitroethane was to employ triethylamine in dimethylsulphoxide, with the nitroalkylisobenzofuranone being obtained in good yield. Reductive cleavage of (174) to the nitroalkylbenzoic acid (175) was accomplished using sodium borohydride in a straightforward manner.<sup>113</sup>

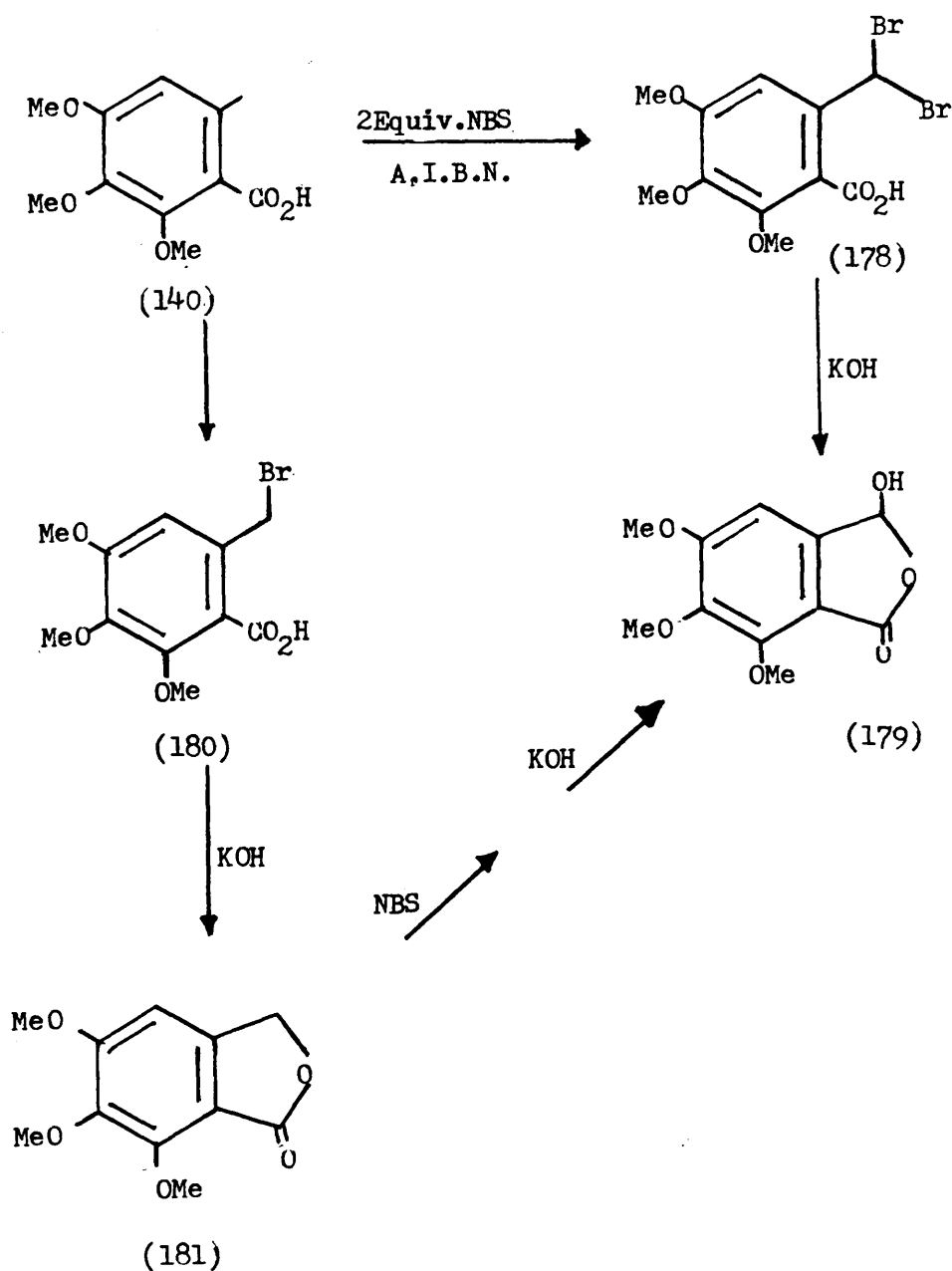


Scheme 41

The major problem encountered by Hauser in this synthetic route was conversion of the nitro functionality to a carbonyl group. McMurray's<sup>114</sup> titanium trichloride procedure produced a mixture of compounds. Oxidative hydrolysis of nitroalkanes to carbonyl groups with hydrogen peroxide and potassium carbonate was also examined but the yields of the benzopyran-1-one obtained from this procedure were low and somewhat erratic.<sup>115</sup> It was also found that the quantity of hydrogen peroxide used had no effect on the yield of product obtained. Experiments were carried out which confirmed that hydrogen peroxide was a non-essential ingredient to the reaction. This result suggested that a simple Nef reaction was taking place. Consequently a modified Nef reaction was carried out in which the anion of (175) was generated with sodium methoxide in methanol, which was then added to a mixture of sulphuric acid in methanol.<sup>116</sup> The product was principally the carboxy ketal (176) which on treatment with acetic anhydride and perchloric acid in refluxing ethyl acetate furnished the isocoumarin (177). The characteristic sky blue colour which is formed on addition of the nitrate anion to the acid solution can be used to follow the progress of the reaction. The disappearance of the blue colour indicates that the reaction is complete.

With this synthesis in mind, a route to the correctly substituted phthalaldehydic acid (179) was needed. If this could be easily made then an efficient synthesis

of 6,7,8-trihydroxyisocoumarin may be possible. It was thought that if dibromination of 2,3,4-trimethoxy-6-methyl benzoic acid (140) could be successfully achieved to give the dibromo acid (178), then hydrolysis of the diacid with potassium hydroxide would furnish the required phthalaldehydic acid (179). 2,3,4-Tri-methoxy-6-methylbenzoic acid (140) had been made previously.



Scheme 42

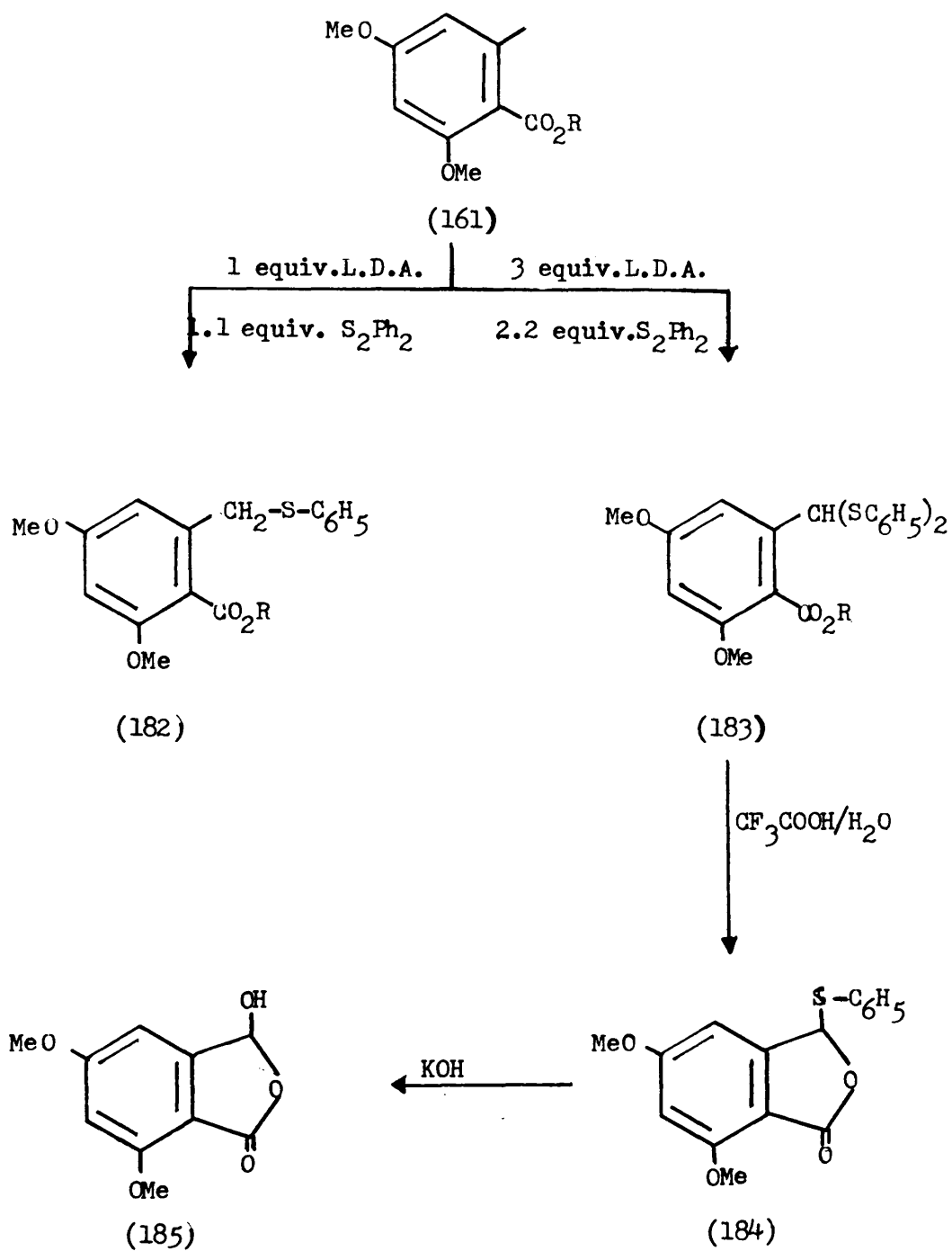
The simplest way to achieve dibromination of (140) was thought to be with N-bromosuccinimide.<sup>117,118</sup> A catalyst is normally required and this can be either ultra-violet light or the free radical generator diazoisobutyronitrile (A.I.B.N.). N-Bromosuccinimide has been employed for many years as a brominating agent for allylic carbons. Its exceptional ability as an allylic brominating agent is presumably due to the polarity of the N-Br bond and also the spatial arrangement of the atoms. It is generally accepted that allylic bromination with N-bromosuccinimide is free radical in nature again being largely due to the polarity of the N-Br bond. In methylated aromatic compounds a maximum of two hydrogen atoms in any one methyl group may be replaced by bromine by the action of N-bromosuccinimide, less if that group is sterically hindered.

Attempts to achieve this allylic dibromination of (140) using two equivalents of N-bromosuccinimide, with ultra-violet light as a catalyst and the free radical generator AIBN failed. The principal compound obtained was the mono alkyl derivative (180) along with a very slight amount of the required dibromo compound (178). However it was thought that this was not a problem since hydrolysis of (180) would give the phthalide (181) which could be brominated again with N-bromosuccinimide as before followed by hydrolysis to give the required hydroxyphthalide (179). (Scheme 42) Further attempts to produce any realistic quantity of the bromo-



alkyl derivative failed with more and more aromatic bromination being observed on each occasion. In the light of these results this method was abandoned. Due to the time factor involved a route to the trimethoxyphthalaldehydic acid was never achieved. However the synthetic route devised by Hauser still has a great deal of potential as a major way of producing the trihydroxyisocoumarin assuming a method of attaining the required trimethoxyphthalaldehydic acid can be found.

A possible method for the synthesis of the required hydroxyphthalide (179) which was looked at but due to boundaries of time was not attempted is the one shown in scheme 43. 3-Thiophenylphthalide (184) has reportedly been synthesised by Hauser and Rhee from the dimethoxy ester (161).<sup>114</sup> Successful mono thiophenylation and monoselenophenylation of the ortho-toluate (161) was clearly accomplished by the addition of (161) to an equivalent of lithium diisopropylamide at  $-78^{\circ}\text{C}$  followed by immediate inverse addition of 1:1 equivalents of diphenyldisulphide to give (182). Treatment of (181) with 3 equivalents of lithium diisopropylamide and 2.2 equivalents of diphenyldisulphide smoothly afforded the dithiophenylated compound (183). This could be partially hydrolysed with trifluoroacetic acid and water to give the 3-thiophenylphthalide (184) in excellent yield. It may be possible that base hydrolysis of (184) would afford the hydroxyphthalide



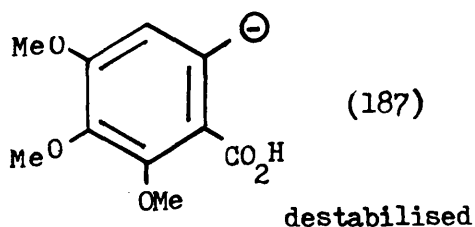
Scheme 43

(185). This route could be used to accommodate the required trimethoxyphthalaldehydic acid (179).

Consequently, this may be a possible way to overcome the problem synthesis of 6,7,8-trihydroxyisocoumarins.

One obvious question which still remains unanswered at the end of this chapter must be, why this trihydroxyisocoumarin (132) is so difficult to synthesise. The 6,8-dihydroxyisocoumarin is relatively easy to make yet 6,7,8-trihydroxyisocoumarin is not obtainable, even though a number of syntheses have been attempted, including those which are well established routes to isocoumarins. Reactions employing both mild and harsh conditions alike were attempted but all as yet have failed to furnish any of the required compound.

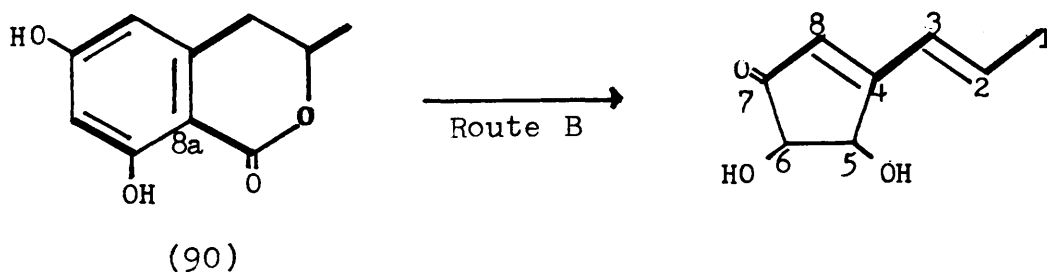
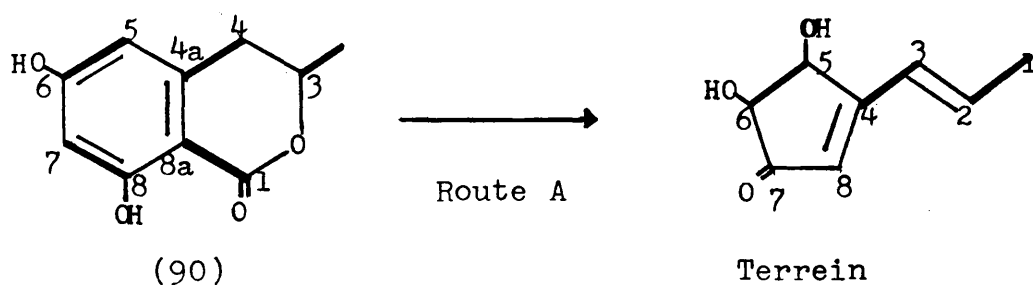
One suggestion as to the continuing failure of the acetylation reaction to yield the trihydroxyisocoumarin must be due to the effect that the extra methoxy group at position 7 of the aromatic ring is having on the compound. Electrons from this methoxy group at position 7 will be pushed into the aromatic ring in order to stabilise a structure such as (187). In some cases it may cause elimination of a functional group which may be required in the reaction thus preventing the required product being formed. However, the reason as to the failure of the production of the isocoumarin largely remains unanswered.



# Chapter 4.

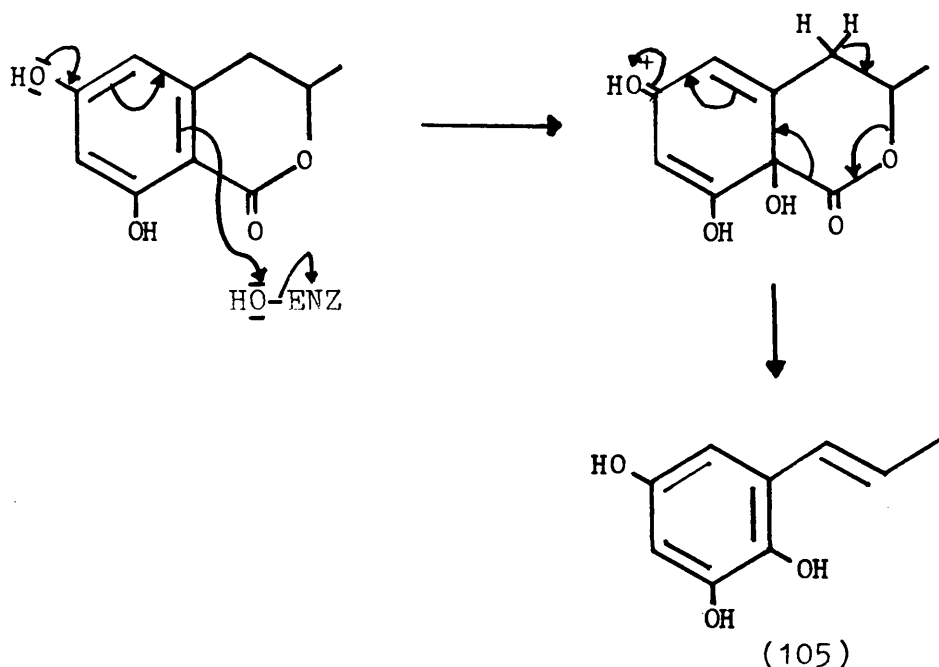
## Synthesis of Phenylpropenes.

The possible intermediacy of the trihydroxy-phenylpropene (105) in the biosynthesis of terrein (21) was investigated. Early work by Hill<sup>64</sup> showed that there were two possibilities for the conversion of the dihydroisocoumarin (90) into terrein. The isocoumarin (90) is either hydroxylated at C-5 (route A), which will then become C-5 of terrein, or the isocoumarin can undergo initial oxidative decarboxylation at C-8a (route B), such that this will become C-5 of terrein.



These two cases would both give different labelling patterns when fed with doubly labelled acetate. The second case agrees with the experimentally determined labelling pattern carried out by Hill. The carboxyl

group cannot be lost before hydroxylation takes place since this would leave a symmetrical intermediate which would cause scrambling of the labels and this is not observed. A possible route involving enzymic oxidation at C-8a of the isocoumarin is as follows:-



Thus, the trihydroxyphenylpropene (105) could be the next intermediate on the pathway to terrein. This prompted an attempt to try and synthesise 2,3,5-trihydroxyphenylpropene (105). This could then be labelled in an appropriate position and fed to Aspergillus terreus.

Terrein contains an exocyclic double bond with E stereochemistry. It is therefore probable that any possible biosynthetic precursors of terrein which contain a propene grouping, will be likely to have double bonds with trans stereochemistry.

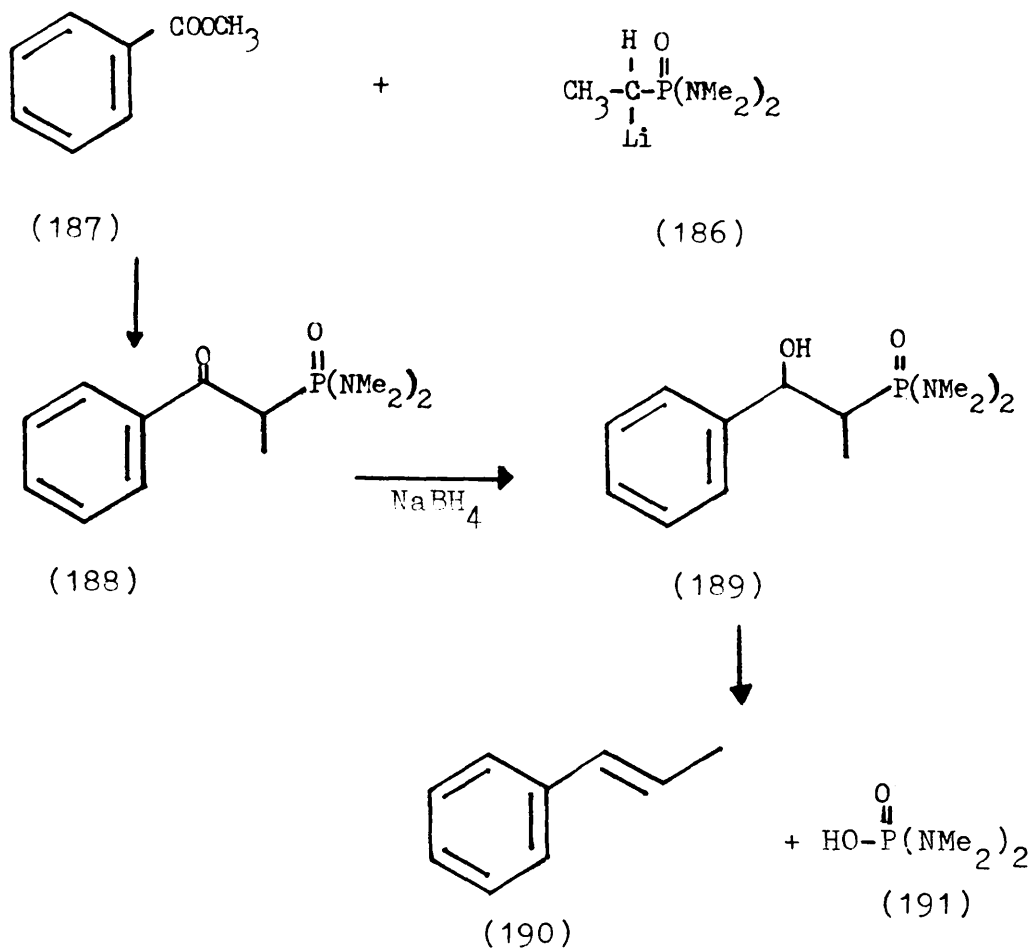
The route chosen for the synthesis of (105) was one adopted by MacLachlan<sup>116</sup> in his synthesis of chlorinated phenylpropenes. A number of major considerations needed to be taken into account in the synthesis of phenylpropenes.

1. The aromatic ring has a high degree of oxygenation and is extremely prone to electrophilic attack.
2. The 2,3,5-trihydroxy substitution pattern in the aromatic ring is not commercially available and thus has to be made synthetically.
3. 2,3,5-Trihydroxyphenyl derivatives are easily oxidised to para-quinones which readily undergo nucleophilic attack.
4. The ring itself is highly substituted which may cause steric problems.
5. Polymerisation of the electron rich double bond readily occurs in an acidic medium.
6. A double bond with trans stereochemistry is required.

The major problem that MacLachlan was faced with was to find a route which led to a phenylpropene with a totally trans double bond. A number of routes were attempted including the widely used Wittig reaction and a modification of this known as the Wadsworth-Emmons reaction. However, on all occasions a mixture of cis and trans isomers or the totally cis

isomer was obtained. Corey<sup>121</sup> in 1968 reported a new synthesis of olefins from carboxyl compounds and phosphonic acid bisamides. It was this route that was eventually adopted by MacLachlan. The route is complementary to the Wittig and Horner-Emmons-Wadsworth methods but has certain major advantages in that a directed synthesis of cis and trans olefins can be achieved. The reaction of  $\alpha$ -lithiophosphonamide derivatives with carboxylic esters affords  $\beta$ -ketophosphonamides which can be reduced to give the corresponding  $\beta$ -hydroxyphosphonamides.

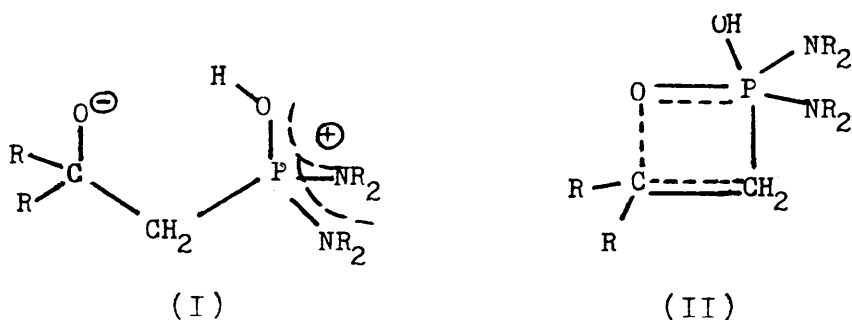
The required carbanionic reagents of type (186) are cleanly and conveniently generated by the reaction of an appropriate alkylphosphonic acid bis(dimethylamide) with one equivalent of butyllithium. Reaction of this  $\alpha$ -lithiophosphonamide has been carried out with a carboxylic ester such as methyl benzoate (187) giving the  $\beta$ -ketophosphonamide (188). Stereoselective reduction of the carbonyl group with sodium borohydride is totally specific giving the  $\beta$ -hydroxyphosphonamide (189) with the hydroxyl bearing carbon having only one configuration. This  $\beta$ -hydroxyphosphonamide will then undergo an acid catalysed cycloelimination reaction in a syn manner to give specifically (1E)-1-phenylprop-1-ene (190) and tetramethylphosphorodiamidic acid (191). (Scheme 44).



Scheme 44

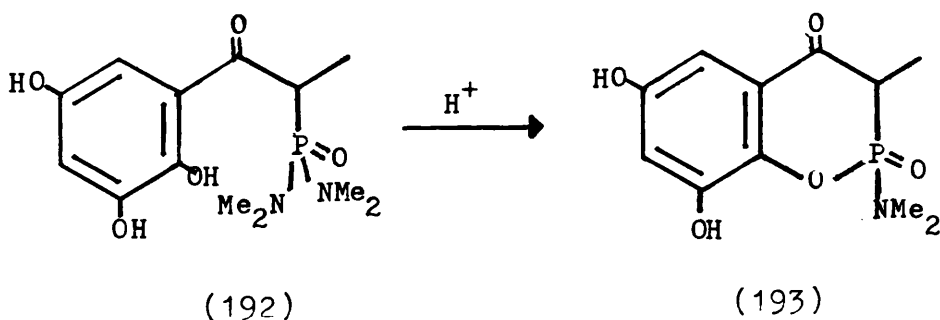
The formation of olefins by thermal decomposition of  $\beta$ -hydroxy phosphonamide bisamides probably involves a zwitter-type intermediate of type (I). This could undergo a syn cycloelimination via a postulated four-membered cyclic transition state of type (II), in which the C-O and C-P bond breaking are well advanced.



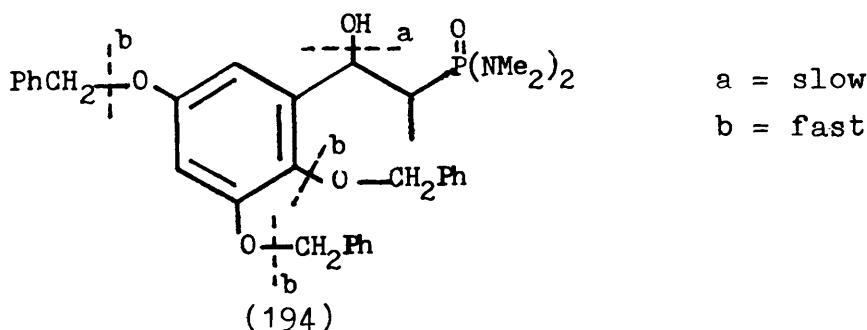


This reaction was repeated on other carboxylic esters such as 3,5-disubstituted methyl benzoates. The reaction was found to be successful when hydroxy substituents were protected as methyl and benzyl ethers. The  $\beta$ -ketophosphonamide and  $\beta$ -hydroxyphosphonamide structures can be regarded as protected olefins thus eliminating the problem of protecting the double bond.

MacLachlan obtained an unusual result when he attempted this reaction on the compound of type (192) whose hydroxyl groups were protected as methyl ethers. On attempts to remove these from the  $\beta$ -ketophosphonamide (192) using boron tribromide an interesting cyclisation reaction occurred giving rise to a compound of type (193). All attempts at reducing this novel ring compound failed.



It was found that protecting the hydroxyl groups as benzyl ethers (194) was more promising. These could be easily removed by hydrogenolysis giving the free phenol which can undergo cycloelimination to give the trans phenylpropene. Noteworthy was the fact that the concurrent rate of hydrogenolysis of the benzylic alcohol function in the  $\beta$ -hydroxyphosphonamide was much slower than the hydrogenolysis of the benzyl protecting groups. Thus it was concluded that it was better to protect the phenol groups as benzyl ethers.



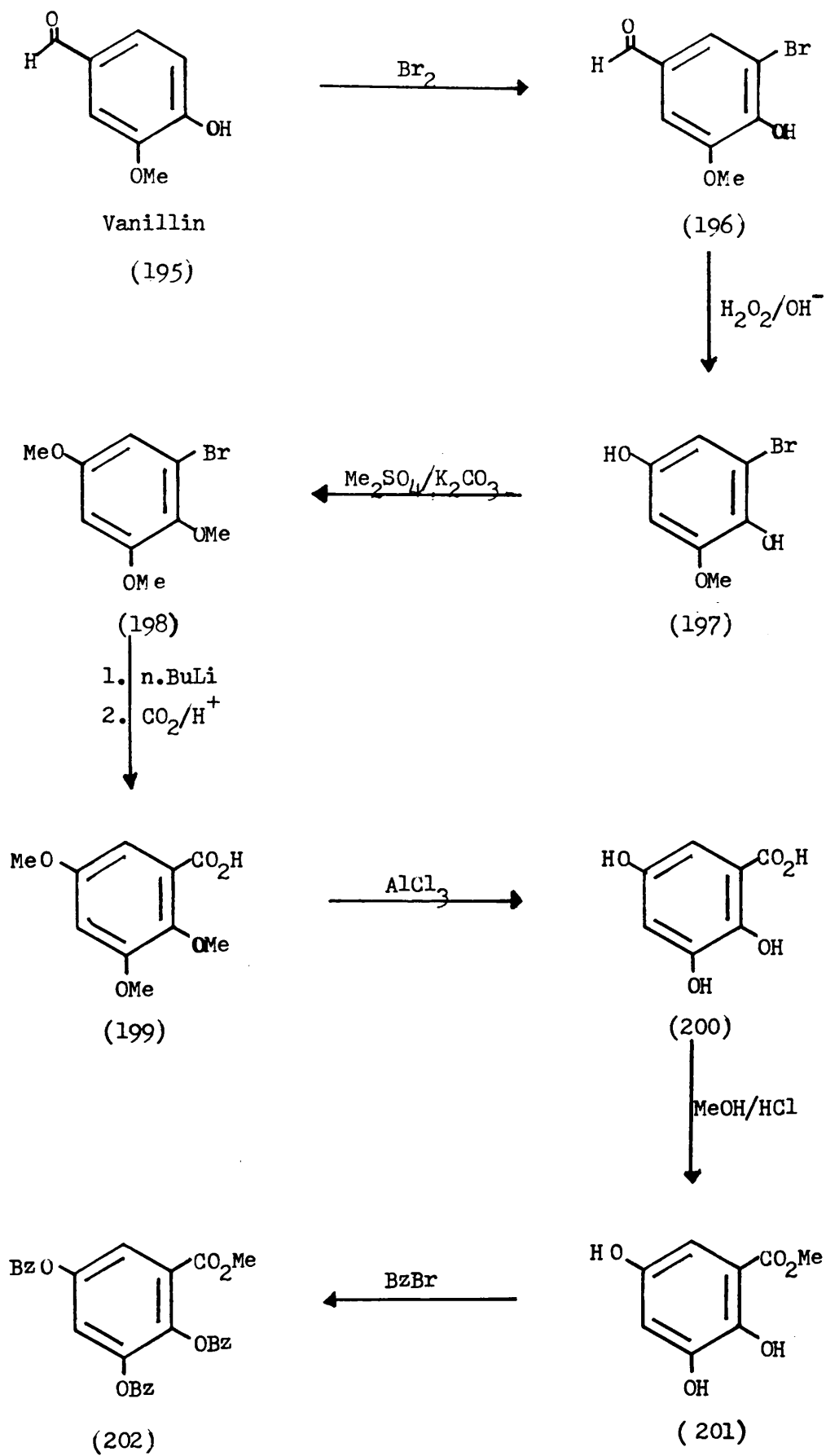
An attempt was made at synthesising 2,3,5-tri-hydroxyphenylpropene (105) by the route shown in scheme 45. Vanillin (195) was brominated in the 5-position using a solution of bromine in glacial acetic acid. 5-Bromo-4-hydroxy-3-methoxybenzaldehyde (196) was obtained as a white crystalline solid in excellent yield. (196) was subjected to a Baeyer-Villiger oxidation which involved converting the aldehydic functionality to a hydroxyl group with the aid of hydrogen peroxide in a basic medium. This afforded the dihydroxy compound (197) of which the

hydroxyl groups were then protected as methyl ethers using dimethylsulphate, affording 2,3,5-trimethoxybromobenzene (198). Carboxylation of the anion formed on the reaction of (198) with n-butyllithium followed by acidification produced the trimethoxybenzoic acid (199). When 2,3,5-trimethoxybenzoic acid (199) was treated with aluminium trichloride in refluxing chlorobenzene removal of the methyl protecting groups was achieved, to give 2,3,5-trihydroxybenzoic acid (200). The deprotected acid (200) was then readily converted into its methyl ester derivative by refluxing a methanolic solution of the acid saturated with dry hydrogen chloride gas. The methyl ester (201) which resulted was then treated with benzyl bromide in order to protect the hydroxyl groups as benzyloxyethers for the forthcoming stages in the synthesis. On reaction of the 2,3,5-tribenzyloxy protected ester (202) with the anion produced by the action of n-butyllithium on ethylphosphonic acid bis(dimethylamide), the  $\beta$ -keto-phosphonamide (203) was produced. Ethylphosphonic acid bis(dimethylamide) (186) was itself formed from the reaction between ethylphosphonic acid dichloride and dimethylamine. Reduction of the ketone group in (203) was achieved using sodium borohydride, which resulted in the production of the  $\beta$ -hydroxyphosphonamide (194). The benzyloxy protecting groups could then be removed by hydrogenolysis with hydrogen gas on a palladium/carbon catalyst giving the free hydroxyl

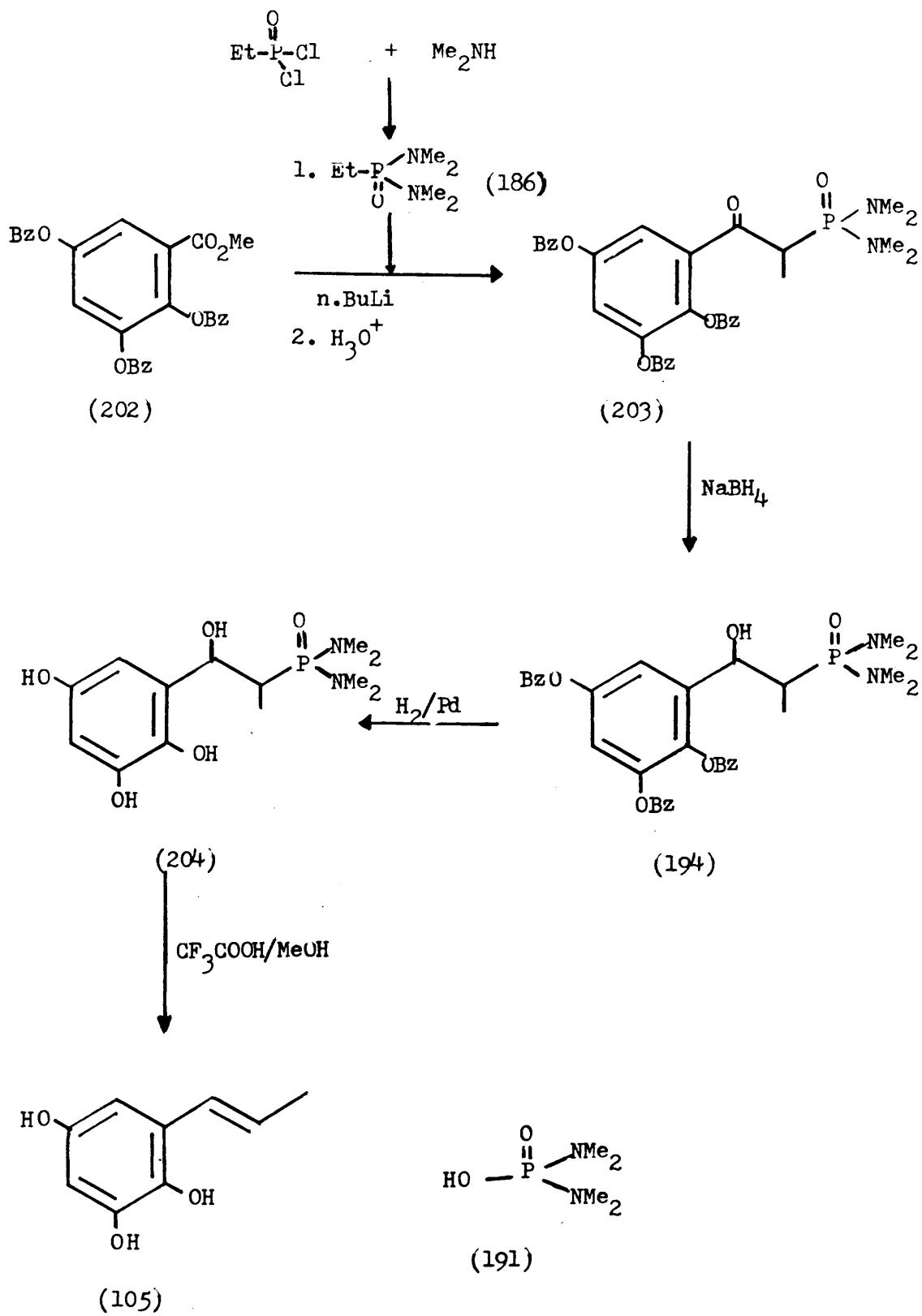
compound (204). Cycloelimination could be achieved successfully by stirring a methanolic solution of the  $\beta$ -hydroxyphosphonamide containing a little trifluoroacetic acid at room temperature. This would result in the production of the byproduct (191) and the required trans 2,3,5-trihydroxyphenylpropene (105).

However, it was found that although the  $\beta$ -hydroxyphosphonamide (194) was successfully made on a small scale, attempts to increase the amount of material being brought through to this stage failed. Hydrogenation of (194) on a small scale did not furnish any of the required compound (204), a black oily solid being the only compound isolated. A  $^1\text{H}$  n.m.r. of this revealed no information on the identity of this compound.

In attempts to scale up the reactions in scheme 45 a large amount of material was taken through to the benzylation step and the material stored at this point until sufficient amounts had been accumulated. This was thought to be a reasonable stage to accumulate material since any excess benzyl bromide left in the reaction had to be distilled off and it was thought better to carry the distillation out in one go. However distillation of the accumulated crude material resulted in removal of the excess benzyl bromide but left behind only a dark brown tar-like substance. A  $^1\text{H}$  n.m.r. of this material revealed that this was indeed the correct compound (202) and appeared to be



Scheme 45



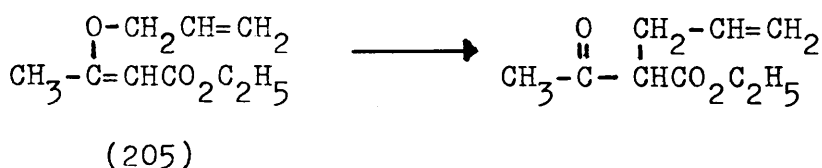
Scheme 45 Contd

relatively free from any impurities. However all attempts at crystallising this material and removing the obvious coloured impurities failed. Distillation of the material was not possible because the boiling point was too high.

One direction of thought was to convert this highly coloured benzyloxylester (202) into its acid derivative in the hope that many of the coloured impurities would be removed. This tribenzyloxyacid could then be re-esterified to give back the ester (202) which would hopefully now be a good deal cleaner. Attempts at hydrolysing the ester (202) with base were successful and a pale yellow crystalline solid was produced. The only problem with this method was that a great deal of material appeared to be lost in the course of the reaction. The crude black tar-like ester (202) was reacted with the  $\alpha$ -lithiophosphonamide (186) but none of the required compound (203) was found to be formed.

Due to the fact that such a lot of material had been lost at the benzylation step and the conversion into the acid derivative this method for producing the trans trihydroxyphenylpropene was abandoned. The above method involved a very long and time-consuming synthesis and due to the boundaries of time a shorter and more efficient approach to the synthesis of 2,3,5-trihydroxyphenylpropene was required.

Allyl ethers of enols and phenols undergo rearrangement to C-alkyl derivatives when heated to sufficiently high temperatures.<sup>122,123</sup> The reaction, named after its discoverer, Claisen in 1912 and now known as the Claisen rearrangement was first observed when O-allylacetoacetate (205) was subjected to distillation at atmospheric pressure in the presence of ammonium chloride.<sup>124,125</sup>



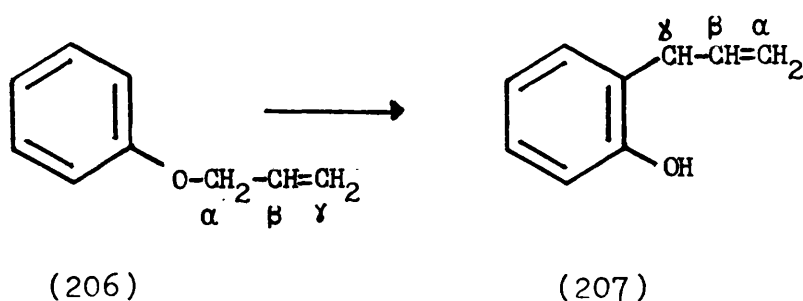
The allyl ethers of phenols rearrange smoothly at temperatures of about 200°C, and in the absence of catalysts. If the ether has an unsubstituted ortho position, then the allyl group will migrate exclusively to this position, the product being the O-allylphenol. If however both ortho positions are blocked then the migration will occur to the para position. Although if both ortho and para positions are occupied then complex decomposition will normally ensue. An allyl group is never seen to migrate to the meta position.

One of the most interesting features of the Claisen rearrangement of allyl phenyl ethers (206) to O-allylphenols (207) is the fact that the carbon atom which becomes attached to the aromatic nucleus is not the same carbon that was attached to the oxygen



atom in the original ether. It is in fact the carbon that was in the  $\gamma$ -position, with respect to the oxygen atom, that becomes attached to the phenyl group.

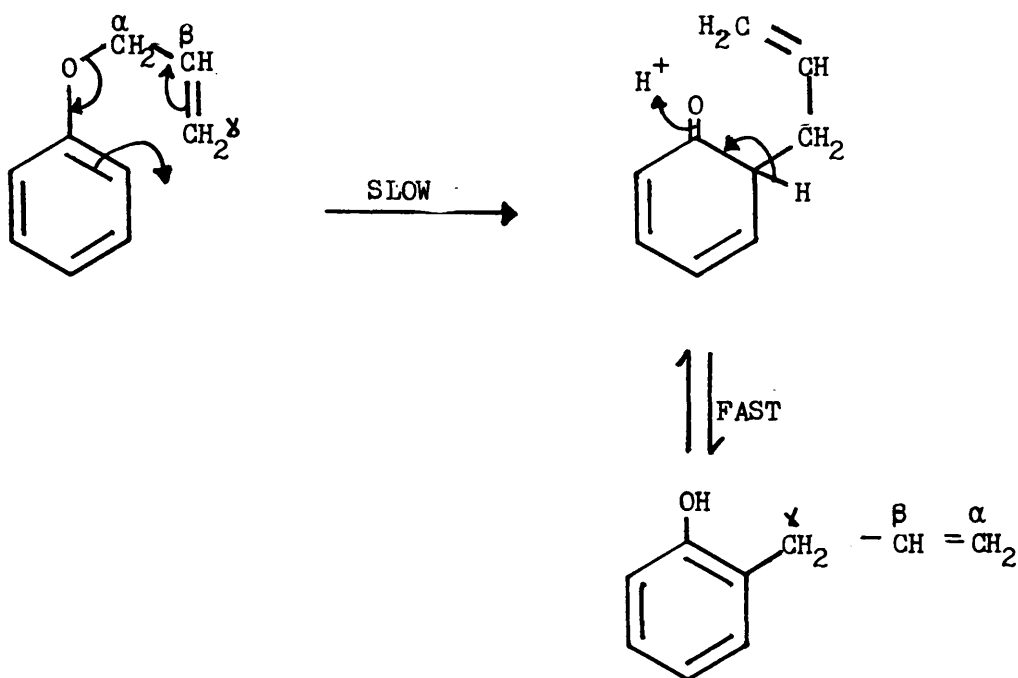
During the rearrangement the double bond of the allyl group shifts from the  $\beta, \gamma$ -position to the  $\alpha, \beta$ -position. The inversion of the allyl group is only apparent when substituents are present on either the  $\alpha$ - or  $\beta$ -carbons.



Any substituents which are attached to the aromatic nucleus do not appear to have any significant effect on the ease of the rearrangement. It is noteworthy that meta directing groups do not hinder the reaction, nor do strongly ortho-para directing groups favour it any more. The Claisen rearrangement to the ortho position is a first order reaction and the process does not require catalysis by acids and bases. The rearrangement is intramolecular and is best represented by the cyclic mechanism indicated in scheme 46.

The breaking of the carbon-oxygen bond and the attachment of the  $\gamma$ -carbon to the ortho position must be simultaneous and it is this step rather than the enolisation step which is the rate-determining one.

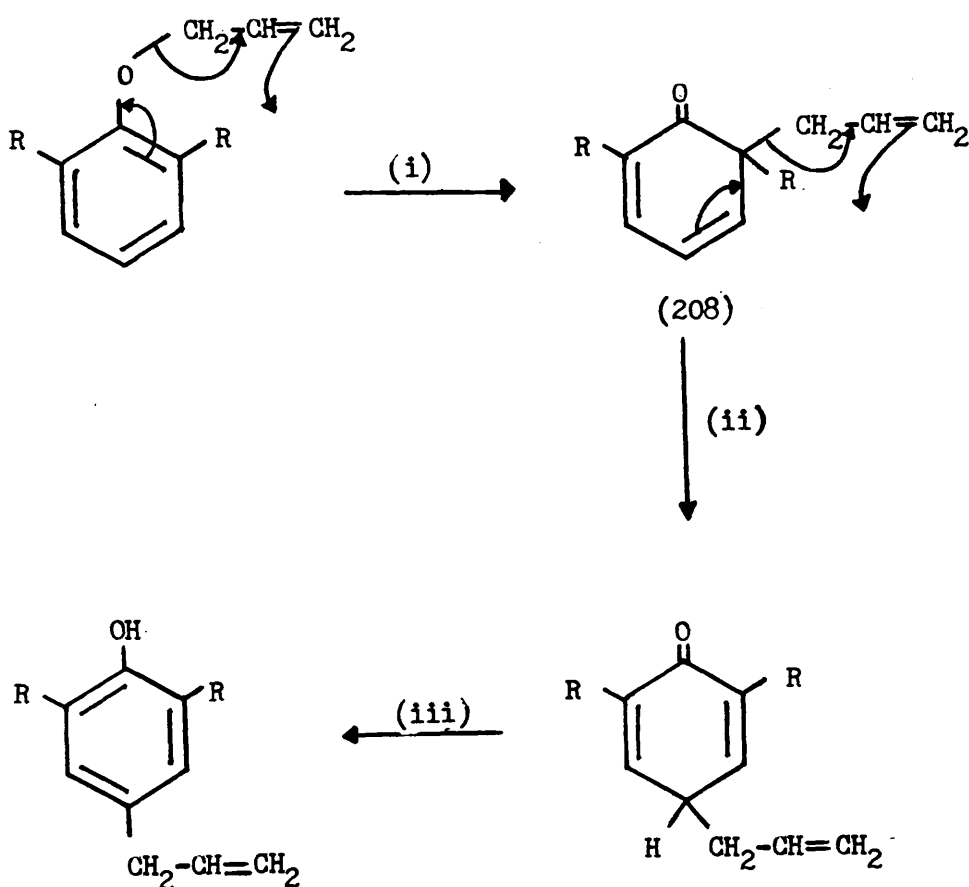
The cyclic mechanism accounts for the occurrence of inversion.



Scheme 46.

Allyl ethers of ortho-disubstituted phenols rearrange to the corresponding para-allyl phenols when both ortho positions are occupied.<sup>126</sup> The para rearrangement is usually just as efficient as the ortho rearrangement but it is noteworthy that it is not usually accompanied by inversion of the allyl group. The para rearrangement is also a first order reaction and the rate is not greatly affected by acids or bases. The non-occurrence of inversion, and the atomic distances involved, make a cyclic mechanism improbable. The fact that para migrations take place under the same conditions as ortho migrations and the close similarity exhibited

by the ortho and para reactions with regard to their energies and entropies of activation indicates that both rearrangements may well have a common (or similar) rate-determining step. The high negative entropy value suggests that this step involves a cyclic transition state. The mechanism of the para-Claisen rearrangement was uncertain for a number of years until Conroy and Firestone<sup>127</sup> provided evidence in support of an intermediate cyclohexandienone (208). (Scheme 47)



Scheme 47.

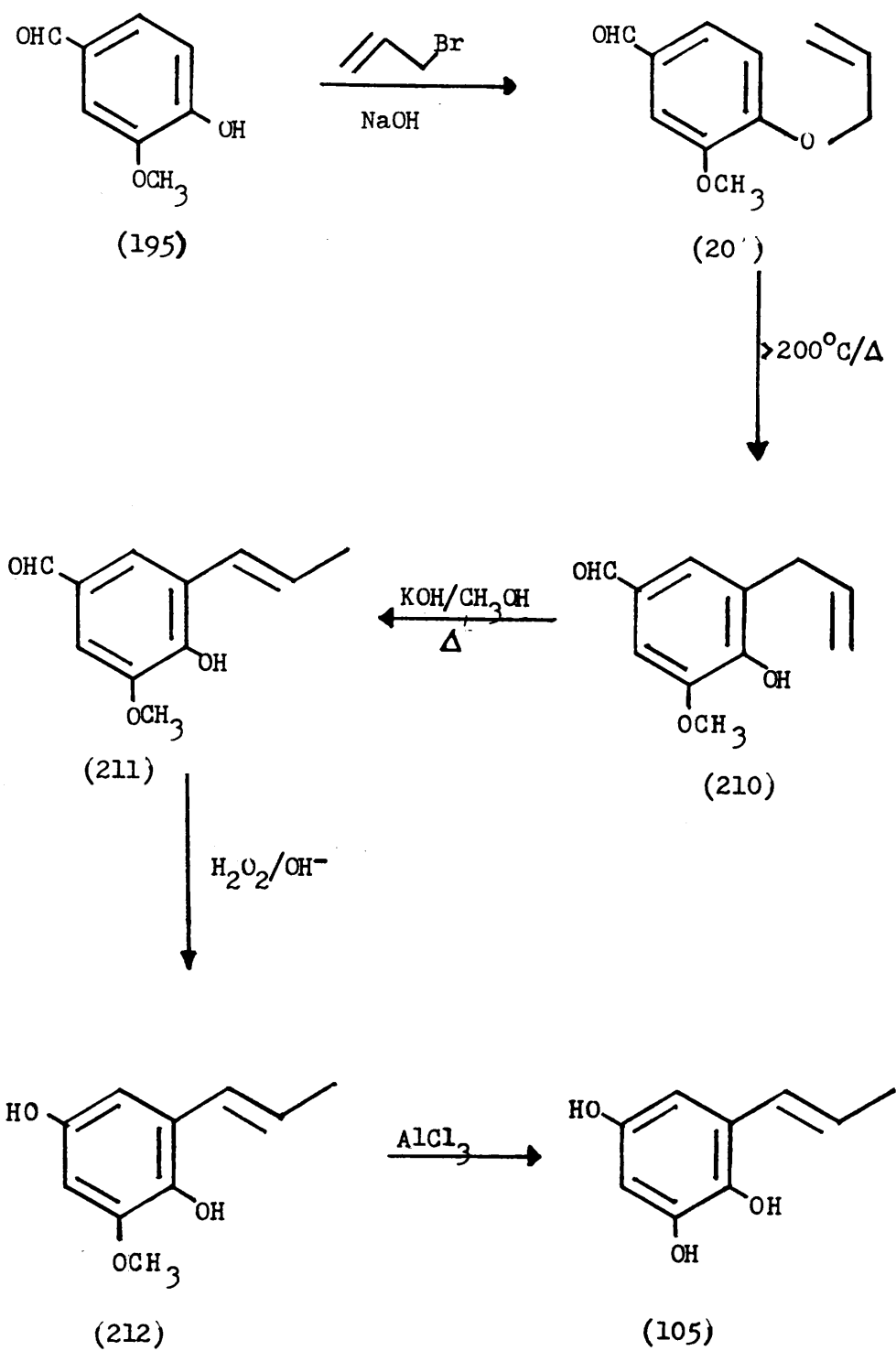
The first step of the para rearrangement involves a prior migration to the ortho position with the production of the dienone (208). The second step of the mechanism is analagous to the first in being a thermal rearrangement of a system in which both an allyl and a vinyl residue are attached to the same atom, although the central atom is carbon rather than oxygen. Decarboxylation of ortho and para carboxy-phenyl allyl ethers has been observed frequently during both ortho<sup>128-132</sup> and para-Claisen<sup>131,132</sup> rearrangements, a result which is predictable from the mechanism seen in scheme 47, since dienones which are either  $\beta$ -keto acids or vinylogous  $\beta$ -keto acids would be involved. Inversion of the allyl group accompanies ortho displacement of carboxyl<sup>129,130</sup> and para displacement occurs without inversion.<sup>131</sup>

It was thus decided that a route involving an ortho-Claisen rearrangement may well be the way to achieve the required trans propenyl side chain of 2,3,5-trihydroxyphenylpropene. Isomerisation of the double bond produced from an ortho rearrangement of O-allyl vanillin is quite readily achieved.

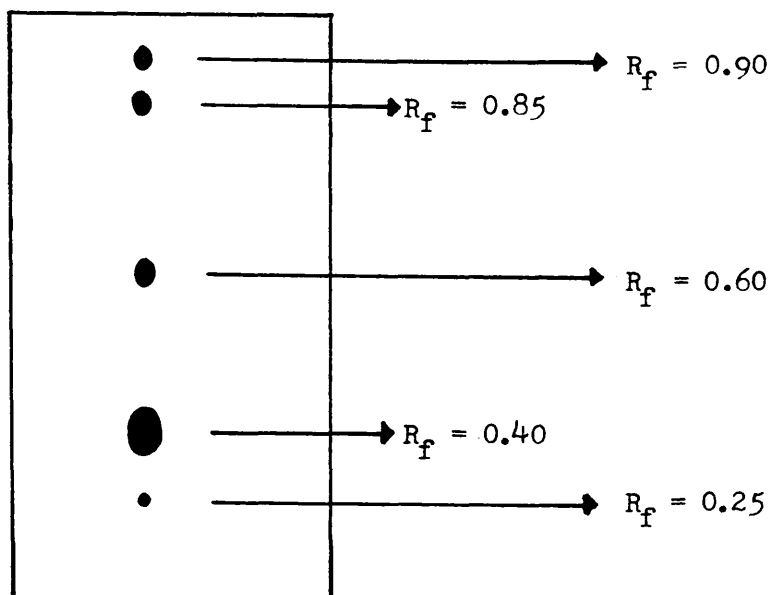
A synthesis was devised that centred round the ortho-Claisen rearrangement. The most suitable starting material was thought to be vanillin (3-methoxy-4-hydroxy-benzaldehyde)(195). This could be converted into the required allylphenyl ether (209) by heating (195) with allyl bromide and sodium hydroxide.

Vanillin allyl ether (209) could then undergo an intramolecular ortho-Claisen rearrangement by heating to a temperature of about 200°C. This thermal rearrangement should furnish 5-allyl vanillin (210). Double bond migration in (210) may be achieved by heating this at reflux in a methanolic solution of potassium hydroxide giving 5-propenyl vanillin (211). A Dakin reaction on (211) could be employed which would oxidise the aromatic aldehyde to a phenol giving (212). Demethylation of the diphenolic compound with aluminium trichloride should afford the required trihydroxyphenylpropene (105) having the correct stereochemistry and substitution pattern<sup>129</sup> (Scheme 48).

This route appears to be much shorter and more efficient than the previous synthesis, affording the required compound in only five steps. The synthetic route was thus attempted. A mixture of vanillin, allyl bromide and a 0.8 M solution of sodium hydroxide were refluxed together for 2 hours.<sup>130</sup> Analysis of the crude product by t.l.c. revealed that five compounds were present with  $R_f$  values of 0.25, 0.40, 0.70, 0.85 and 0.90. Column chromatography allowed efficient separation of these five compounds and their structures were determined spectroscopically.



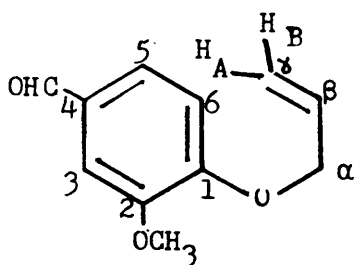
Scheme 48.



The main fraction corresponding to a t.l.c.  $R_f$  value of 0.40 was the required allyl vanillin (209) in a 34% yield. Mass spectrometric analysis showed a parent ion at  $m/z$  192. No reaction with ferric chloride was observed indicating that no phenol was present. A 90 MHz  $^1\text{H}$  n.m.r. showed the characteristic singlet at  $\delta$  9.81 indicating the presence of an aldehyde. A multiplet at  $\delta$  7.38 is due to the hydrogens at the 3- and 5-positions of the aromatic ring. A doublet at  $\delta$  6.79 can be seen with a coupling constant of 9 Hz, this being due to the hydrogen at the 6-position of the aromatic ring. A double doublet of triplets was observed in the range  $\delta$  6.20- $\delta$  5.80 which can be attributed to the proton attached to the  $\beta$ -carbon of (209). This proton is split by the two hydrogens attached to the  $\alpha$ -carbon with a coupling constant of

5.3 Hz. However, it is also split by the two hydrogens attached to the  $\gamma$ -carbon, both differing in the value of the coupling constant due to the cis and trans arrangement of these two protons in relation to the hydrogen at the  $\beta$ -carbon. This  $\beta$ -proton is split by the trans proton  $H_A$  with a large coupling constant of 17.25 Hz and also by the other proton attached to the  $\gamma$ -carbon,  $H_B$  with a cis coupling constant of 10 Hz. The two protons attached to the  $\gamma$ -carbon,  $H_A$  and  $H_B$ , are seen as two double quartets.  $H_A$  appears at  $\delta$  5.29 this is split by the proton attached to the  $\beta$ -carbon with a trans coupling constant of 17.3 Hz. There is also some small fine splitting observed of 1.6 Hz due to long range coupling with the two protons attached to the  $\alpha$ -carbon.  $H_B$  is found at  $\delta$  5.17 with a coupling constant of 10.4 Hz due to splitting by the hydrogen at the  $\beta$ -carbon and a coupling constant of 1.4 Hz due to the two protons at the  $\alpha$ -carbon. The two protons attached to the  $\alpha$ -carbon are split firstly by the proton at the  $\beta$ -carbon with a coupling constant of 5.3 Hz. Long range allylic coupling is also observed with the two protons attached to the  $\gamma$ -carbon to the value of 1.5 Hz. Finally a large singlet can be seen at  $\delta$  3.74. This is due to the methoxy group in (209). From this data it can be concluded that the fraction corresponding to an  $R_f$  value of 0.4 is the required compound, allyl vanillin (209) and has the structure shown on the next page.

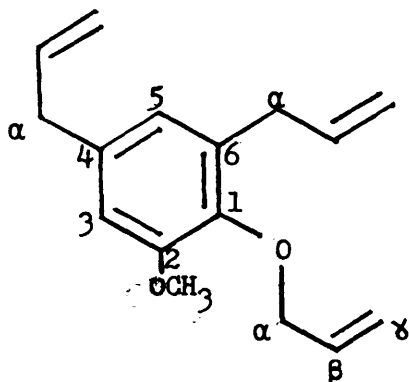




(209)

The spot corresponding to an  $R_f$  value of 0.9 was shown by mass spectrometry to have a parent ion of  $m/z$  244. This compound did not show any colour change with ferric chloride indicating that no phenol was present. The 90 MHz spectrum showed no aldehydic protons to be present. A large singlet at  $\delta$  7.62 was observed with intensity 2. This indicates that only two aromatic protons are now present, both appearing in the same position in the n.m.r. spectrum. This peak may be due to the hydrogens at position 3 and 5 of the aromatic ring, since they will not experience the strong deshielding effect of the aldehyde group which appears not to be present in this compound. H-6 which was split into a doublet by the ortho proton at H-5 appears to have disappeared. A complex multiplet is seen in the range  $\delta$  6.35- $\delta$  5.70. Another very complicated set of signals is present from  $\delta$  5.45- $\delta$  4.90 which may be due to the O-allyl ether group and also some rearrangement of this group. A double doublet is seen to be centred at  $\delta$  4.45 which is thought to be due to the protons at the  $\alpha$ -carbon of the allyl ether grouping,  $OCH_2-CH=CH_2$ . Two overlapping doublets can be seen, one centred at  $\delta$  3.40 and the other at  $\delta$  3.32, with an

intensity of 4. These could be due to the two protons attached to the  $\alpha$ -position of the allyl group. It is therefore a possibility that the four hydrogens are due to two allyl groups attached to the 4- and 6-positions of the aromatic ring. The position of these protons in the n.m.r. spectrum are indicative of a  $\alpha$ -CH<sub>2</sub> of an allyl group attached to an aromatic nucleus. The structure indicated below was proposed for this fraction.



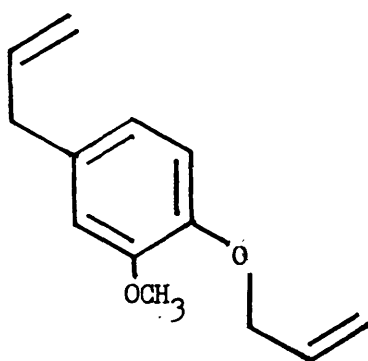
(213)

The fraction corresponding to an  $R_F$  value of 0.85 has a  $m/z$  value of 204. A 90 MHz spectrum shows no characteristic aldehyde peak. A multiplet at  $\delta$  6.80 of intensity 3 is due to the aromatic protons at positions -3, 5 and 6.

The hydrogens at positions 3 and 5 have been shifted upfield, since they will not experience the strong deshielding effect of the aldehydic group which does not appear to be present in this compound.

A multiplet from  $\delta$  4.35 -  $\delta$  3.80 is seen and this may be due to protons attached to the  $\beta$ -position of the allyl group. The most noticeable feature of this spectrum

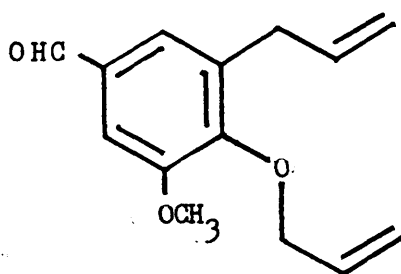
was a broad doublet at  $\delta$  4.66 of intensity 2. This is indicative of an  $\text{Ar-OCH}_2\text{CH=CH}_2$  grouping. Another doublet at  $\delta$  3.35 was also observed and this gives further indication of a  $\text{Ar-CH}_2\text{CH=CH}_2$  grouping. Thus it appears that an O-allyl group and an Ar-allyl group are both present in this compound. The structure proposed for this fraction is (214) seen below.



(214)

The fraction corresponding to an  $R_f$  value of 0.6 was shown by mass spectroscopy to have a parent ion of  $m/z$  232. No reaction with ferric chloride was observed indicating no phenol to be present. A 90 MHz n.m.r. spectrum showed a characteristic aldehyde peak at  $\delta$  9.88. A singlet at  $\delta$  7.34 was observed and by comparing with the authentic vanillin allyl ether spectrum it was thought that this is due to two aromatic protons at position 3 and 5 of the aromatic ring. Again it was noticed that the 6 position was occupied due to the non-appearance of the doublet in (209). Some rearrangement of the O-allyl group must again be

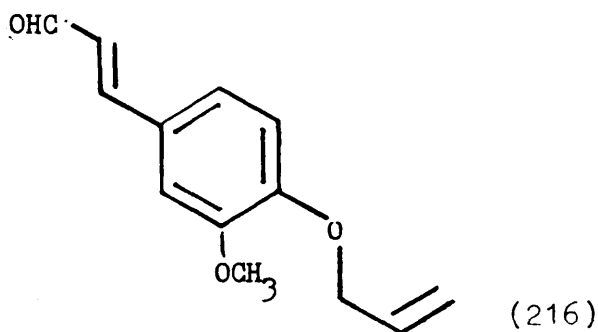
occurring. Also of note in this spectrum was the doublet of intensity 2 at  $\delta$  4.59 and the doublet at 3.45 also of intensity 2. These indicate the presence of both the  $\text{OCH}_2\text{CH}=\text{CH}_2$  grouping and its rearranged product  $\text{Ar}-\text{CH}_2-\text{CH}=\text{CH}_2$  respectively. The following structure shown below is proposed (215).



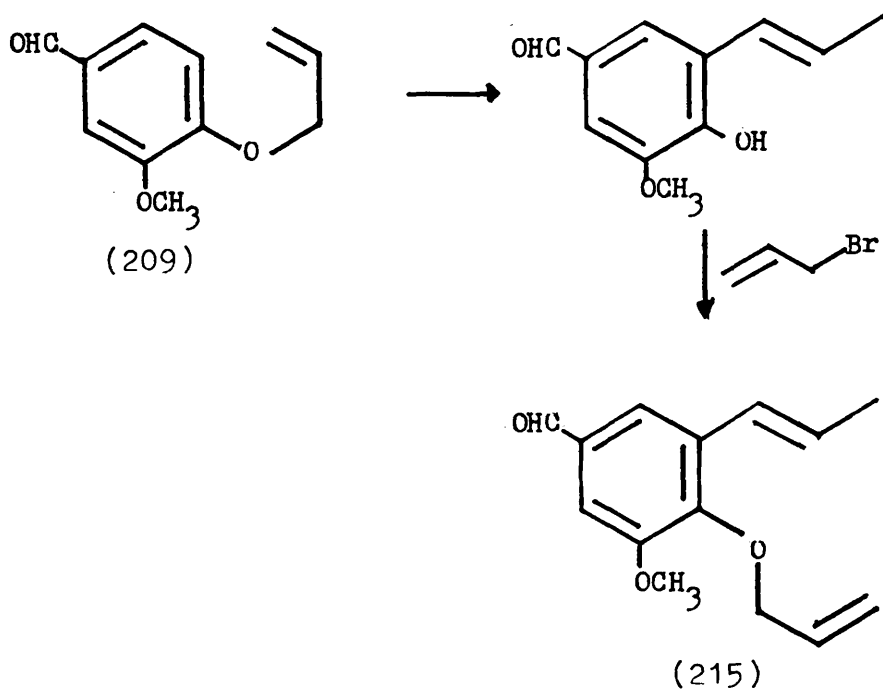
(215)

Finally the fraction with an  $R_f$  value of 0.25 was analysed. This had a molecular ion with  $m/z$  218. No reaction with ferric chloride was observed, thus no phenol is present in this molecule. The 90 MHz  $^1\text{H}$  n.m.r. spectrum showed that this compound was contaminated with a little vanillin allyl ether, probably from the previous fraction. A doublet at  $\delta$  9.88 of intensity 1 could be seen. The aromatic protons appear as a very complicated multiplet but on elimination of those due to vanillin allyl ether they appear very characteristic of an  $\alpha,\beta$ -unsaturated aldehyde. This would account for the presence of the doublet at  $\delta$  9.44 with a coupling constant of 7 Hz. A doublet at  $\delta$  4.67 is characteristic of the  $\text{OCH}_2\text{CH}=\text{CH}_2$  group of vanillin allyl ether. No doublet around the

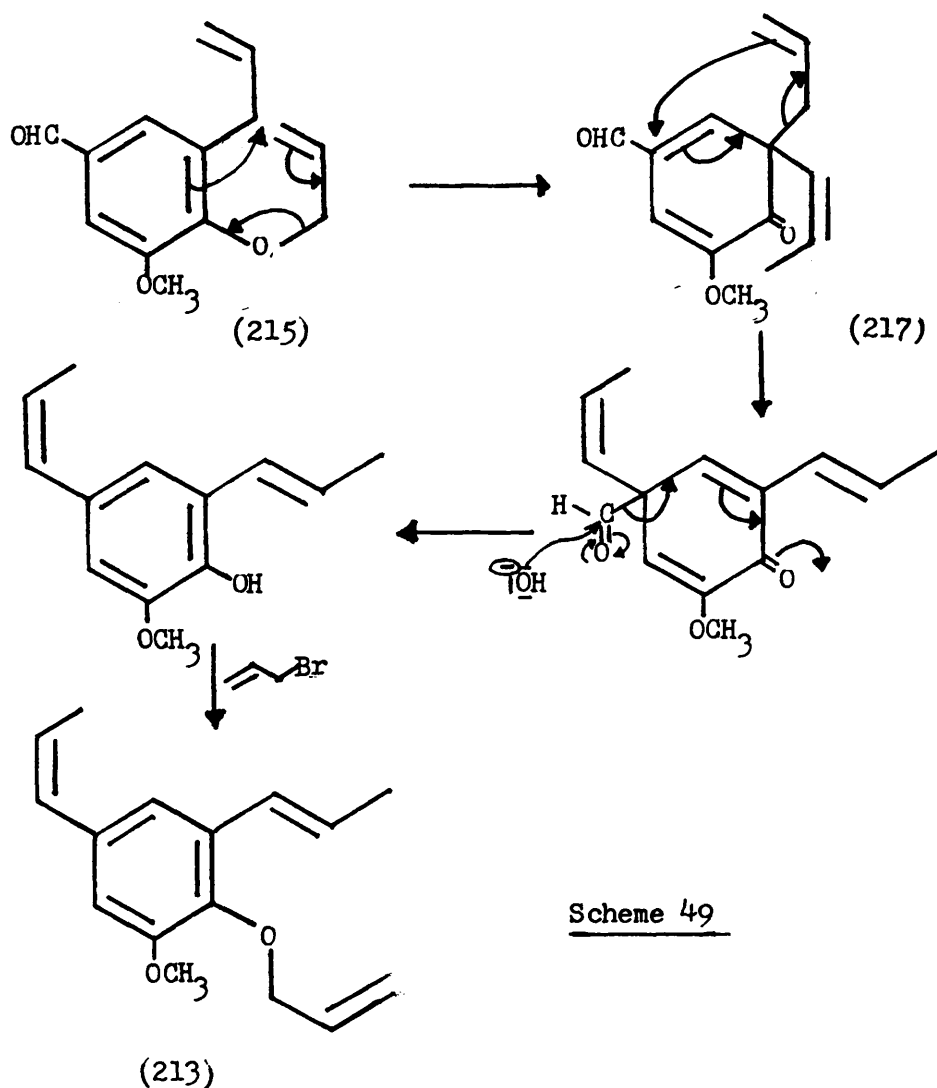
3.3 range can be seen in the spectrum suggesting that no  $\text{ArCH}_2\text{-CH=CH}_2$  grouping is present. The unusual product shown below is suggested as the structure for the compound with an  $R_f$  of 0.25.



The unexpected additional products produced from the reaction of vanillin with allyl bromide may be due to further rearrangement of the major product vanillin allyl ether (209). If vanillin allyl ether underwent an ortho-Claisen followed by further addition of another molecule of allyl bromide then this would account for one of the unexpected products (215).

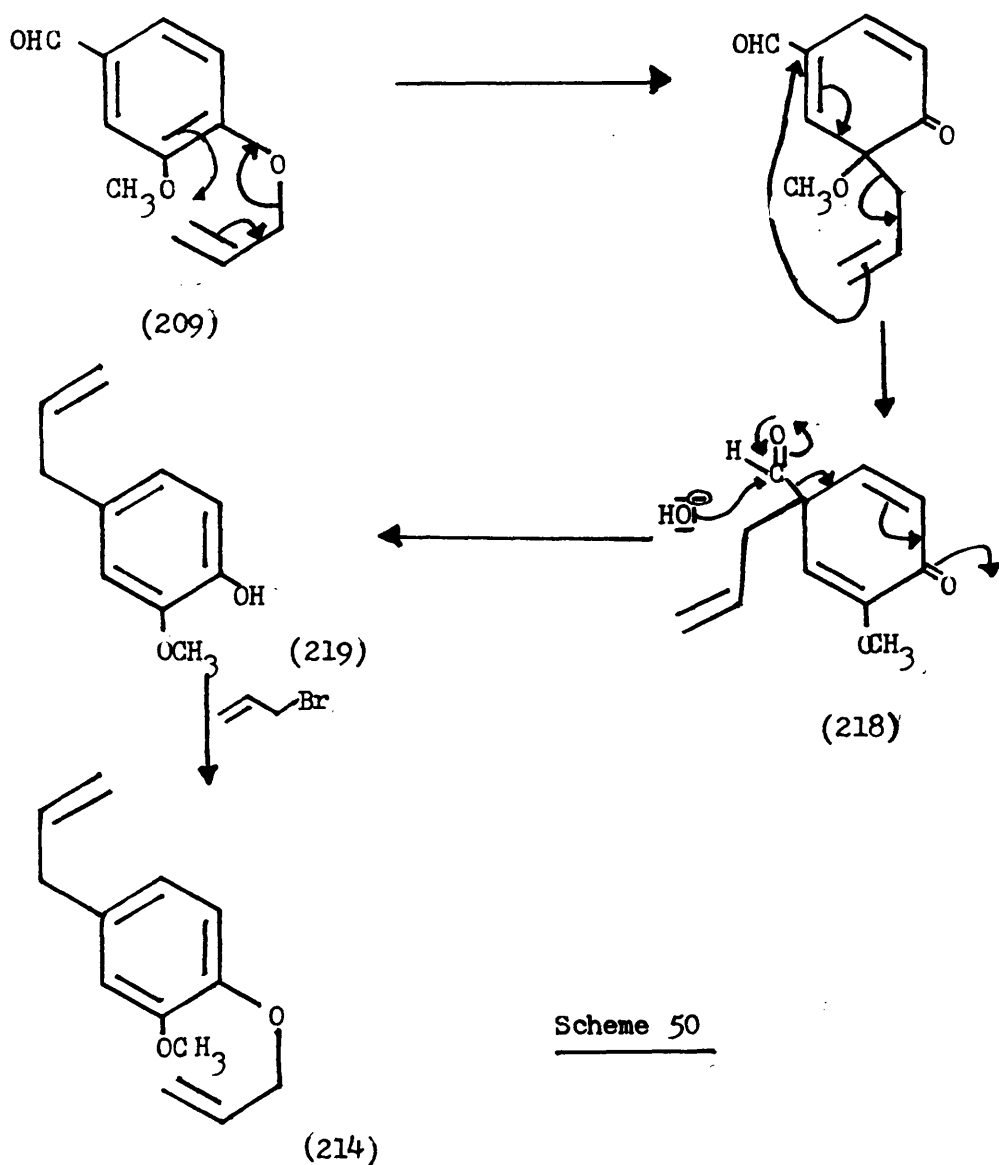


If the product (215) was to undergo a further para-Claisen rearrangement then another of the unexpected products (213) would be formed. The mechanism for the formation of (213) is outlined in scheme 40. If (215) undergoes a prior migration to one of its ortho positions then an intermediate such as (217) would be formed. This is very sterically crowded thus resulting in one of the allyl groups rearranging to the para position. The aldehydic group attached at this position can be easily lost by attack of an hydroxide ion on the carbonyl group followed by decarboxylation. This will relieve any steric hindrance, thus accounting for another of the proposed structures (213).



Scheme 49

Another of the compounds isolated from the reaction mixture was (214). This could have arisen by a similar mechanism to the one shown above. A para-Claisen rearrangement of (209) could occur even though one of the ortho positions is vacant. The reaction must be occurring in the opposite direction giving an intermediate of type (218). Attack of hydroxide ions, which are present in the reaction mixture on the carbonyl group followed by decarboxylation would lead to compound (219). Addition of another molecule of allyl bromide would furnish the required product (214). (Scheme 50)



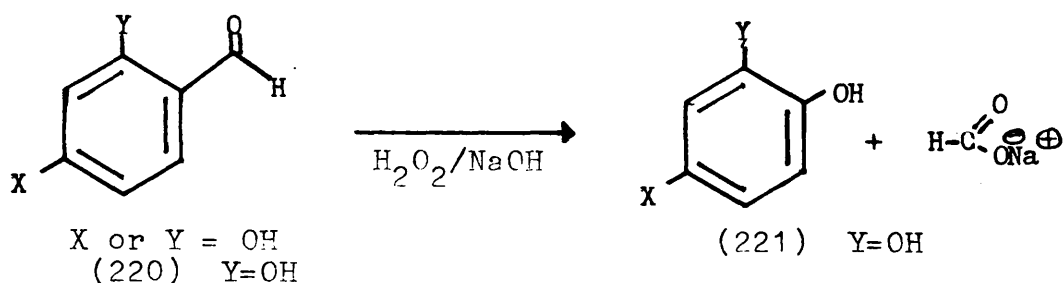
Scheme 50

The pure vanillin allyl ether obtained from the previous reaction was heated with the high boiling point solvent, N,N-diisopropylaniline to a temperature above 200° for 15 minutes.<sup>134</sup> Work up followed by t.l.c. analysis revealed five spots. The required Claisen rearranged compound, allyl vanillin (210) was found to be present in the third spot with an  $R_f$  value of 0.35. The five compounds were separated using column chromatography and allyl vanillin was obtained in a 20% yield. The other four compounds were not characterised due to lack of material. Allyl vanillin (210) was then dissolved in a solution of methanolic potassium hydroxide and heated at reflux for 6 hours. Work up afforded the required thermally rearranged product 5-propenyl vanillin in a 93% yield. A small amount of two minor compounds was observed in a t.l.c. analysis of the crude product. However column chromatography allowed efficient separation and 5-propenyl vanillin (211) was obtained in pure form.

An aqueous solution of 5-propenyl vanillin in sodium hydroxide was then treated with hydrogen peroxide by the method of Pew.<sup>134</sup> T.l.c. analysis of the crude reaction mixture showed only starting material to be present. The reaction was repeated several times but on no occasion was any of the required oxidised product (212) found to be present. This prompted a more in depth look at the conditions and mechanism associated with the Dakin reaction.



In 1909 Dakin<sup>135</sup> reported that alkaline hydrogen peroxide oxidised ortho or para-hydroxy aromatic aldehydes to the corresponding dihydroxyaromatic. When the hydroxyl and carbonyl groups occupy a meta position with respect to each other, no such reaction is found to take place.

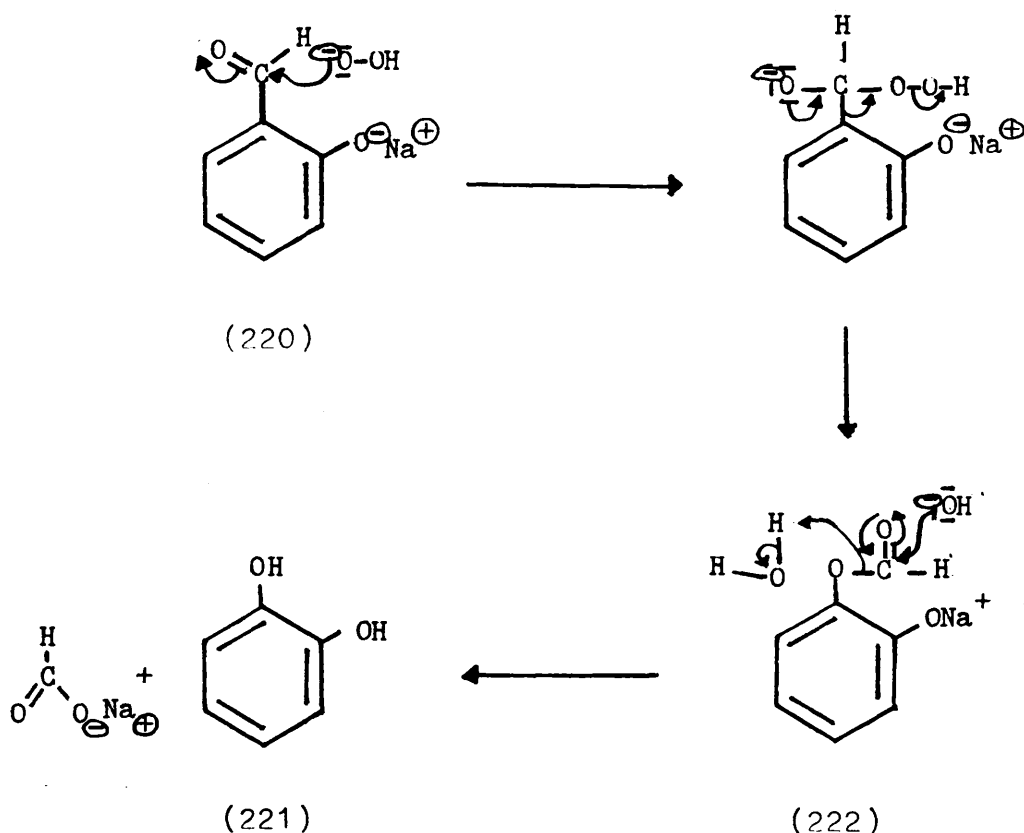


The Dakin reaction has been applied successfully to a variety of substituted phenolic aldehydes. One of the more well documented examples of the Dakin reaction being the conversion of salicylaldehyde (221) into the catechol (222) by alkaline hydrogen peroxide. The Dakin reaction has been curiously reviewed. Mechanistic interpretations have been drawn from the related Baeyer-Villiger oxidation of carbonyl-labelled p-methoxyacetophenone, in which it was found that all the label was retained in the carbonyl group. Hocking used the optimised procedure for the oxidation of salicylaldehyde as a starting point for his study of the Dakin oxidation of o-hydroxyacetophenone.<sup>136</sup> He found that doubling the ratio of sodium hydroxide to hydrogen peroxide in the reaction mixture significantly increased the rate of the reaction. It was also noted

that in those reactions where conditions were such that the pH remained high (above 10.5) then the reaction itself was fast and high yields of the oxidised product were obtained.

The pH effect can be explained by considering the hydrogen peroxide anion concentration. The initial step of the Dakin reaction probably involves attack of a hydroperoxide anion at the carbonyl carbon. Hence the higher the concentration of hydroperoxide anion the faster the oxidation should proceed. Another factor which may contribute to the higher rates observed at high pH could be the fact that a greater proportion of dissociated phenol will be present in the substrate being oxidised. Phenyl migration is also thought to be more facile in an intermediate which contains an ionised hydroxyl group than in an intermediate where the hydroxyl group is protonated.

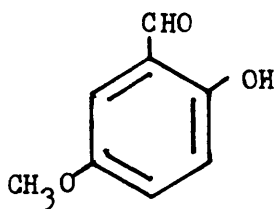
The mechanism of the Dakin reaction (Scheme 50) is thought to involve initial attack of a hydroperoxide anion on the carbonyl carbon in (220). Phenyl migration will then result since this is the most electron rich species giving the intermediate (222) which then undergoes hydrolysis to furnish the dihydroxy-aromatic compound (221).



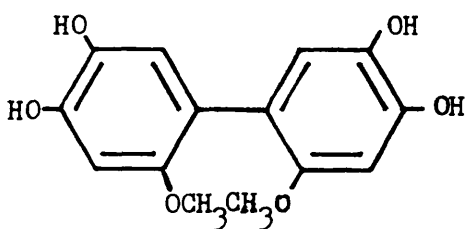
Scheme 51.

There are some isolated examples of the oxidation of the normal products produced by the Dakin reaction. In one such example the oxidation of 2-hydroxy-5-methoxybenzaldehyde (223) led to the formation of an abnormal product.<sup>137</sup> When hydrogen peroxide was added to an aqueous solution of 2-hydroxy-5-methoxybenzaldehyde and sodium hydroxide, a precipitate was formed which softened at 200°C and decomposed at 300°C. The experiment was repeated using lithium and potassium hydroxides but it was observed that regardless of the hydroxide used; if the reaction mixture was allowed

to stand for several days the same precipitate was the only isolable product. Rosenblatt suggested that due to its insolubility in ether and its high melting point that the compound was not a simple nuclearly substituted catechol but the bis-(4-methoxycatechol) (224) or an isomer of this.



(223)



(224)

One thought as to the reason for the failure of the Dakin oxidation on 5-propenyl vanillin (211) could be that the aromatic ring in (211) is extremely electron rich due to the electron donating substituents attached to the ring. Thus ring oxidation may ensue rather than the required oxidation of the aldehyde, giving highly oxidised products.

If further study of this reaction is to be carried out then there is perhaps the possibility that other peroxide reagents could be tried.<sup>138</sup> Possibilities include perbenzoic acid, peracetic acid, monoperphthalic acid or maybe even hydrogen peroxide in a different solvent such as ether. It should also be noted that in alkaline solution hydrogen peroxide decomposes relatively rapidly and is particularly sensitive to impurities. These facts should be given sufficient

consideration and care taken to ensure that sufficient excess of the reagent is available.

## Chapter 5

### EXPERIMENTAL

#### General Procedures.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Ultra-violet spectra were measured on a Pye Unicam SP8-100 spectrophotometer. Infra-red spectra were recorded for potassium bromide discs (unless otherwise stated) on a Perkin-Elmer 580 spectrometer. The following abbreviations are used : s - strong; m-medium; w-weak and br-broad.

Proton nuclear magnetic resonance spectra were determined on a Perkin-Elmer R32 (90 MHz) spectrometer using deuteriochloroform as solvent (unless otherwise stated). Tetramethylsilane was used as an internal standard.  $^1\text{H}$  N.m.r. spectra were also recorded at 200 MHz on a Bruker WP200SY spectrometer, using a deuterium lock system. The chloroform ( $\text{CHCl}_3$ ) in  $\text{CDCl}_3$  was set at  $\delta$  7.25 p.p.m. as internal standard. The following abbreviations are used : s-singlet; d-doublet; t-triplet; q-quartet; m-multiplet; dd-double-doublet; dt-double-triplet; dq-double-quartet and br-broad. Carbon nuclear magnetic resonance spectra were recorded on a Bruker WP200SY spectrometer, setting the reference  $\text{CDCl}_3$  signal at  $\delta$  77.0 p.p.m.

Mass spectra were obtained using a VE/Kratos MS12 spectrometer or a VG/Kratos MS90S spectrometer for high resolution.

All apparatus and solutions involved in the growth of the micro-organism were sterilised before and after use in an autoclave.

Preparative layer chromatography was carried out using Merck Kieselgel GF<sub>254</sub> on plates (20 x 20 cm) coated with silica gel of 0.1 mm thickness. Analytical thin layer chromatography (t.l.c.) was carried out on commercial plates with a 0.25 mm layer of the same silica gel.

Solvents were dried in the following manner : Tetrahydrofuran (THF) was distilled from sodium/benzophenone; methanol and ethanol were dried using magnesium activated with iodine and stored over 3A molecular sieves; acetone and butanone were dried using anhydrous potassium carbonate and stored over molecular sieves; and ether was dried using sodium wire.

Organic solvents were dried over anhydrous magnesium sulphate (unless otherwise stated) and evaporated under reduced pressure using a rotary evaporator.

### Culture and Harvesting of *Aspergillus terreus*.

Stock cultures of *Aspergillus terreus* (International Mycological Institute No. 44339) were maintained on potato-dextrose agar slants. The slants were inoculated with a spore inoculum from the master strain and incubated at 25°C for 14 days after which time they were used as inocula for large-scale cultures or stored at 4°C.

Large scale cultures of *Aspergillus terreus* were grown as surface cultures in Roux bottles on a Czapek Dox medium consisting of glucose (50 g), sodium nitrate (2 g), potassium dihydrogen phosphate (1 g), potassium chloride (0.5 g), magnesium sulphate (0.5 g), ferrous sulphate (0.01 g) and deionised water (1L). The medium was first sterilised by autoclaving for 20 min. at 120°C and 15 psi. The vessels were inoculated by mycelium transfer and grown at 25°C with subdued light for 5 days followed by strong light for a further 9 days (total culture period 14 days). On day 14 the broth was decanted from the mycelium and continuously extracted at end pH with ethyl acetate for 24 h.

### Isolation of Terrein.

The ethyl acetate extract was evaporated to half volume, dried over anhydrous magnesium sulphate, then further evaporated to leave a semi-solid brown material. The brown extract was chromatographed on a 20 x 20 cm plate of 0.75 mm thickness using



GF<sub>254</sub> silica gel and ethyl acetate as eluent. Terrein ( $R_f$  0.6) was isolated as a yellow solid which was recrystallised from acetone as fine pale-yellow needles m.p. 126-127.5°C (lit.<sup>54</sup>, 127°C).

$\nu_{\max}$  3400 m, 1695 s, 1630 s and 1565 s  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  200 MHz  $(\text{CD}_3)_2\text{CO}$  6.80 (1H, dq,  $\underline{J}$  7 Hz and 16 Hz, 2-H), 6.41 (1H, d,  $\underline{J}$  16 Hz, 3-H), 5.94 (1H, s, 8-H), 4.98 (1H, br s, OH), 4.71 (1H, br s, 5-H), 4.07 (1H, s, 6-H), 1.88 (3H, d,  $\underline{J}$  7 Hz,  $\text{CH}_3$ ) p.p.m;  $\delta_{\text{C}}$  200 MHz  $[(\text{CD}_3)_2\text{CO}]$  206.45 (s, C=O), 169.14 (s, C-4), 140.16 (d, C-2), 126.25 (d, C-3), 125.72 (d, C-8), 82.22 (d, C-6), 77.74 (d, C-5), 19.33 (q, C-1) p.p.m; m/z 139 ( $\text{M}^+ - \text{Me}$ ).

#### Feeding Experiments.

The isocoumarins (107) and (108) were fed as their sodium salts to Aspergillus terreus on day 7 and the fungal broth extracted on day 14. The isolation of the feeding experiments are discussed in Chapter 3A.

#### Ethyl 1,2-dihydro-o-orsellinate <sup>139</sup>(112)

Ethyl acetoacetate (91.5g; 89.6 ml) was added dropwise to a stirred solution of sodium (18.25g) in dry ethanol (500ml) at such a rate a gentle reflux was maintained. The solution was heated at reflux for a further 30 min, then ethyl crotonate (79.8g; 86.9ml) was added dropwise during 15 min. The mixture was heated at reflux for 6 h, then stirred at room temperature for a further 16 h. The sodium salt of the enolate which was produced was

filtered off and washed with dry ether and air dried. The dried salt was then dissolved in water and acidified with dilute hydrochloric acid. The aqueous solution was extracted with ether, dried and evaporated to give ethyl 1,2-dihydro-o-orsellinate as an oil which slowly crystallised on standing. The product was recrystallised from benzene/hexane to give pale yellow crystals (74.85g, 54%), m.p. 87-89°C (lit., <sup>140</sup> 89-90°C);  $\nu_{\text{max}}$  3190 br, 1735 s, 1630 s, 1570  $\text{m cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 7.18 (1H, s, OH), 5.49 (1H, s, 3-H), 4.24 (2H, q,  $\underline{J}$  7Hz,  $\text{OCH}_2\text{CH}_3$ ), 3.08 (1H, d,  $\underline{J}$  10Hz, 1-H), 2.81-2.05 (3H, m, 5- and 6-H), 1.28 (3H, t,  $\underline{J}$  7Hz,  $\text{OCH}_2\text{CH}_3$ ), 1.05 (3H, d,  $\underline{J}$  7Hz,  $-\text{CHCH}_3$ ) p.p.m;  $m/z$  196 ( $\text{M}^+$ ).

Ethyl 3,5-dibromo-o-orsellinate<sup>141</sup> (113)

A solution of bromine (35.5 g, 11.35 ml) in glacial acetic acid (15 ml) was added with stirring to a solution of ethyl 1,2-dihydro-o-orsellinate (12.5g) in glacial acetic acid (100 ml) at such a rate that the temperature of the reaction mixture remained above 60°C. The reaction mixture was stirred for 16 h then poured onto iced water (500 ml); a white precipitate of ethyl 3,5-dibromo-o-orsellinate separated and was collected by filtration, washed with water and air-dried. A portion was recrystallised from dichloromethane/hexane (13.6g, 61%), m.p. 104-105°C (lit., <sup>141</sup> 105-106°C);  $\nu_{\text{max}}$  3495 br, 1565 s, 1215  $\text{br cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 4.44 (2H, q,  $\underline{J}$  7Hz,  $\text{OCH}_2\text{CH}_3$ ), 2.65 (3H, s, Ar- $\text{CH}_3$ ), 1.40 (3H, t,  $\underline{J}$  7Hz,  $\text{OCH}_2\text{CH}_3$ ) p.p.m;  $m/z$  352, 354 ( $\text{M}^+$ )

Ethyl-o-orsellinate (114)

Ethyl 3,5-dibromo-o-orsellinate (10 g) was dissolved in sodium hydroxide solution (2M, 120 ml) and Raney nickel alloy (10 g) was added portionwise with stirring at 0°C. After complete addition of the alloy, the mixture was stirred at 0°C for a further hour, then filtered through a pad of Celite. The filtrate was poured onto ice-cold concentrated hydrochloric acid (75 ml) and the acidic solution extracted with ether (3 x 25 ml). The ethereal extracts were dried and evaporated to give the product as a thick brown oil of which only a little crystallised from glacial acetic acid (10.5g, 10%), m.p. 128-130°C (lit., <sup>142</sup> 131-133°C);  $\nu_{\max}$  3370 br, 1640 s, 1585 m cm<sup>-1</sup>;  $\delta[(\text{CD}_3)_2\text{CO}]$  11.60 (1H, s, OH), 8.98 (1H, s, OH), 6.21 (2H, s, 2xAr-H), 4.47 (2H, q,  $\underline{J}$  7Hz,  $\text{OCH}_2\text{CH}_3$ ), 2.46 (3H, s, Ar-CH<sub>3</sub>), 1.38 (3H, t,  $\underline{J}$  7Hz,  $\text{OCH}_2\text{CH}_3$ ) p.p.m; m/z 196 (M<sup>+</sup>).

2,4-Dimethoxy-6-methylbenzoic acid. (115)

To a solution of ethyl-o-orsellinate (0.5 g) in anhydrous acetone (10 ml) was added dimethylsulphate (0.75 ml) and anhydrous potassium carbonate (1.25 g). The mixture was heated at reflux for 8 h with stirring. On cooling the reaction mixture was filtered and the precipitate washed with acetone after which the organic solution was evaporated leaving a dark brown oily residue. The residue was dissolved in ether and washed with aqueous ammonia solution (10%, 3x6 ml), sodium hydroxide solution (10%, 3x5 ml) and water

(3 x 5ml). The ethereal solution was evaporated to give a pale yellow oil which was then suspended in potassium hydroxide solution (10%, 7.5 ml) and heated at reflux for 6h. On cooling, the basic solution was washed with ether (3 x 5 ml) then acidified with concentrated hydrochloric acid at 0°C. The aqueous solution was extracted with ethyl acetate (4 x 10 ml) dried and evaporated to give the required compound which was recrystallised from dichloromethane/hexane (0.16 g, 28%), m.p. 139°C (lit.,<sup>64</sup> 140°C);  $\nu_{\max}$  1680 s, 1600 s cm<sup>-1</sup>;  $\delta$  (CDCl<sub>3</sub>) 6.39 (2H, m, 2xAr-H), 3.91 (3H, s, OMe), 3.82 (3H, s, OMe), 2.51 (3H, s, Ar-CH<sub>3</sub>) p.p.m; m/z 196 (M<sup>+</sup>).

Ethyl 2,4-diacetoxy-6-methylbenzoate.<sup>143</sup>

Ethyl 1,6-dihydro-o-orsellinate (9.9 g) was dissolved in a mixture of glacial acetic acid (30 ml) and acetic anhydride (15 ml) by warming and the resultant solution cooled to 12°C. A solution of bromine (8 g, 2.57 ml) in acetic acid (5 ml) was then added dropwise at this temperature with stirring.

A small portion of the resultant solution was poured into cold dilute hydrochloric acid and the suspension extracted with ether. The ethereal solution was washed repeatedly with water and brine and then dried and evaporated to give ethyl 3-bromo-o-orsellinate.  $\delta$  (CDCl<sub>3</sub>) 9.30 (2H, brs, 2xOH), 6.75 (1H, m, Ar-H), 4.15 (2H, q,  $J$  7Hz, OCH<sub>2</sub>CH<sub>3</sub>), 2.17 (3H, s, ArCH<sub>3</sub>), 1.20 (3H, t,  $J$  7Hz, OCH<sub>2</sub>CH<sub>3</sub>) p.p.m.

The bulk of the reaction mixture was treated in the following

manner. A rapid stream of dry nitrogen was bubbled through the solution as the temperature was raised slowly, the solution was then heated at reflux for 2 h. After cooling the solution was washed repeatedly with water and brine, then dried and evaporated to give the required product as a colourless oil of which only a very slight amount crystallised from light petroleum 40-60° C (0.7 g, 5%), m.p. 40-42°C. (lit., <sup>144</sup> 42°C)  $\delta$  (CDCl<sub>3</sub>) 6.70 (2H, m, 2xAr-H), 4.41 (2H, q, J 7Hz, OCH<sub>2</sub>CH<sub>3</sub>), 2.50 (3H, s, Ar-CH<sub>3</sub>), 2.30 (3H, s, COCH<sub>3</sub>), 2.23 (3H, s, COCH<sub>3</sub>), 1.40 (3H, t, J 7Hz, OCH<sub>2</sub>CH<sub>3</sub>) p.p.m.

3,5-Dimethoxytoluene<sup>145</sup> (118)

Orcinol (37.2 g) dissolved in dry acetone (375 ml) containing anhydrous potassium carbonate (165 g) and dimethylsulphate (57 ml) was heated at reflux for 8 h with the aid of mechanical stirring. After cooling, the mixture was filtered and the residue washed with acetone (2 x 150 ml). The combined organic solutions were evaporated to give an oil which was dissolved in ether (300 ml). The organic layer was washed with an ammonia solution (10%, 4 x 75 ml), sodium hydroxide solution (10%, 3 x 60 ml) and water (3 x 60 ml). The organics were dried and evaporated to give an oil which was purified by distillation (30.53 g, 80.5%), (50°C/0.1 mmHg);  $\nu_{\max}$  1610  $\text{cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 6.27 (3H, s, Ar-H), 3.69 (6H, s, 2 x OMe), 2.21 (3H, s, Ar-CH<sub>3</sub>) p.p.m;  $m/z$  152 ( $\text{M}^+$ )

2-Bromo-3,5-dimethoxytoluene<sup>146</sup> (119)

Bromine (9.6 ml) in 1,2-dichloroethane (60 ml) was added with stirring during 5 min to a solution of 3,5-dimethoxytoluene (30 g) in 1,2-dichloroethane (300 ml). Stirring was continued for a further 20 min at room temperature. The organic solution was washed with water (3 x 120 ml), dried and evaporated to give an oil which slowly crystallised on standing. The product was recrystallised from methanol as cubes (23.26 g, 51%), m.p. 57°C (lit.<sup>7</sup> 57°C);  $\nu_{\max}$  1585  $\text{cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 6.40 (1H, d,  $J$  3Hz, Ar-H), 6.30 (1H, d,  $J$  3Hz, Ar-H), 3.81 (3H, s, OMe), 3.79 (3H, s, OMe), 2.34 (3H, s, Ar-CH<sub>3</sub>) p.p.m;  $m/z$  232, 230 ( $\text{M}^+$ ).

2,4-Dimethoxy-6-methylbenzoic acid<sup>147</sup> (115)

n-Butyllithium (25.3 ml, 1.6M in hexane) was added to a stirred solution of 2-bromo-3,5-dimethoxytoluene (10 g) in THF (250 ml) at  $-78^{\circ}\text{C}$  under nitrogen. The solution was stirred at this temperature for 15 min and then poured onto an excess of crushed dry ice. The solution was allowed to warm to room temperature and water (125 ml) was added. The organic solution was evaporated and the remaining aqueous solution washed with ether (3 x 125 ml). Acidification with dilute hydrochloric acid followed by extraction with ethyl acetate (3 x 100 ml), drying and evaporating yielded the desired compound which was recrystallised from acetone (5.5 g, 65%), m.p.  $138-140^{\circ}\text{C}$  (lit.<sup>47</sup>  $140^{\circ}\text{C}$ );  $\nu_{\text{max}}$  2950 br,  $1690\text{ s cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 6.35 (2H, brs, 2 x Ar-H), 3.90 (3H, s, OMe), 3.80 (3H, s, OMe), 2.50 (3H, s, Ar- $\text{CH}_3$ ) p.p.m;  $m/z$  196 ( $\text{M}^+$ ).

3,5-Dimethoxyhomophthalic acid<sup>46</sup> (116)

n-Butyllithium (50.6 ml, 1.6M in hexane) was added to a solution of diisopropylamine (8.2 g) in THF (25 ml) under nitrogen at  $0^{\circ}\text{C}$  with stirring. After 10 min the solution was cooled to  $-78^{\circ}\text{C}$  and a solution of 2,4-dimethoxy-6-methylbenzoic acid (4 g) and dimethylcarbonate (4.4 g) in THF (25 ml) was added dropwise during 15 min. The cooling bath was removed and the solution allowed to warm to room temperature. After 4 h, water

water (30 ml) was added and the suspension stirred for 16 h.

The organic solvents were removed by evaporation and the resulting aqueous solution washed with ether (2 x 20 ml). After acidification with dilute hydrochloric acid, the aqueous solution was extracted with ethylacetate (3 x 20 ml). The combined extracts were dried and evaporated to give 3,5-dimethoxyhomophthalic acid (3.36 g, 69%), m.p. 167-170°C (from ethanol-hexane) (lit.,<sup>148</sup> 172-173°C);  $\nu_{\max}$  3500-2800 br, 1725 s, 1700 s, 1605 s, 1575 s  $\text{cm}^{-1}$ ;  $\delta$  [(CD<sub>3</sub>)<sub>2</sub>CO] 6.57 (2H, s, Ar-H), 3.88 (3H, s, OMe), 3.82 (3H, s, OMe), 3.75 (2H, s, CH<sub>2</sub>) p.p.m; m/z 240 (M<sup>+</sup>).

2,4-Dimethoxy-6-(2-oxopropyl)benzoic acid (120)

3,5-Dimethoxyhomophthalic acid (1.6 g) was added portion-wise to a mixture of acetic anhydride (3.2 ml) and dry pyridine (0.8 ml) at such a rate it all dissolved. After 5 min, dry ether (12 ml) was added to the thick precipitate which had formed and stirring continued for 2 h. Sodium hydroxide solution (120 ml, 4M) was slowly added and the mixture refluxed until all the solid material had dissolved. The solution was cooled and washed with an equal volume of dichloromethane, then cautiously acidified. The acidic solution was extracted with ethyl acetate (3 x 30 ml), dried and evaporated to give the required compound which was recrystallised from acetone (1.03 g, 65%), m.p. 138-140°C (lit.,<sup>65</sup> 140°C);

$\nu_{\max}$  3385 s, 1690 s, 1605 s, 1580 s  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  200 MHz



$[(\text{CD}_3)_2\text{CO}]$  6.54 (1H, d,  $J$  2Hz, Ar-H), 3.83 (6H, s, 2 x OMe),  
2.10 (3H, s,  $-\text{COCH}_3$ ) p.p.m;  $m/z$  238 ( $\text{M}^+$ )

6,8-Dimethoxy-3-methylisocoumarin (122)

2,4-Dimethoxy-6-(2-oxopropyl)benzoic acid (2 g) was dissolved in a solution of acetic anhydride and perchloric acid in ethyl acetate (200 ml) made up by the method of Edwards and Rao<sup>71</sup> as follows : A solution of ethyl acetate (50 ml) and perchloric acid (0.05 ml, 72%) was added to a second solution of ethyl acetate (120 ml) containing acetic anhydride (19.2 ml). The combined solutions were made up to 200 ml with ethyl acetate giving a reagent which was 1M in acetic anhydride and  $10^{-3}\text{M}$  in perchloric acid.

The solution was allowed to stand at room temperature for 10 min then washed with water (3 x 150 ml) and a solution of sodium hydrogencarbonate (3 x 100 ml). The organics were then dried and evaporated to give a brown solid which was purified by column chromatography using dichloromethane as eluent and recrystallised from ethanol (1.23 g, 67%), m.p. 155-156°C (lit.,<sup>149</sup> 156-159°C);

$\nu_{\text{max}}$  1710  $\text{s cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 6.40 (1H, s, Ar-H), 6.28 (1H, s, Ar-H), 6.15 (1H, s, 4-H), 4.92 (3H, s, OMe), 4.85 (3H, s, OMe), 2.19 (3H, s,  $\text{CH}_3$ ) p.p.m;  $m/z$  200 ( $\text{M}^+$ ).

3,4-Dihydro-6,8-dimethoxy-3-methylisocoumarin

6,8-Dimethoxy-3-methylisocoumarin (120 mg) in dry ethyl acetate (20 ml) was hydrogenated at room temperature in the presence of a 10% palladium on charcoal catalyst. When the uptake of hydrogen had ceased (approx. 20 ml) the catalyst was removed by filtering through Celite. The organic solvent was then evaporated to give the desired product which was recrystallised from ether/hexane as needles (110 mg, 91%), m.p. 125-126°C (lit.<sup>11</sup> 125-126°C);  $\nu_{\max}$  1715 br, 1600 s, 1580 m  $\text{cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 6.39 (1H, d,  $J$  2Hz, Ar-H), 6.29 (1H, br s, Ar-H), 4.50 (1H, m, 3-H), 3.90 (3H, s, OMe), 3.87 (3H, s, OMe), 2.80 (2H, d,  $J$  7Hz,  $\text{CH}_2$ ), 1.43 (3H, d,  $J$  7Hz,  $\text{CH}_3$ ) p.p.m; m/z 222 ( $\text{M}^+$ )

[1',1',3',3',3'- $^2\text{H}_5$ ]-2,4-Dimethoxy-6-(2'-oxopropyl)benzoic acid (124)

6,8-Dimethoxy-3-methylisocoumarin (1 g) was added to a solution of sodium (0.53 g) in deuterium oxide (7 ml). The mixture was stirred until all the solid had dissolved, then acidified with deuterium chloride. The aqueous solution was extracted with ethyl acetate, dried and evaporated to give the required product (0.83 g, 81%), m.p. 138 -140°C;  $\nu_{\max}$  3360 br, 1690 s, 1600 s, 1590 s  $\text{cm}^{-1}$ ;  $\delta$  ( $\text{CDCl}_3$ ) 6.50 (1H, d,  $J$  2Hz, Ar-H), 6.40 (1H, d,  $J$  2Hz, Ar-H), 3.96 (3H, s, OMe), 3.83 (3H, s, OMe) p.p.m; m/z 244 ( $\text{M}^+$ ).

[ $4,\alpha,\alpha,\alpha\text{-}^2\text{H}_4$ ]-6,8-Dimethoxy-3-methylisocoumarin (125)

[ $1',1',3',3',3'\text{-}^2\text{H}_5$ ]-2,4-Dimethoxy-6-(2'-oxopropyl)benzoic acid (700 mg) was cyclised to the isocoumarin by the same method described for the unsubstituted compound (440 mg, 68%) same data as unsubstituted compound;  $\delta$  ( $\text{CDCl}_3$ ) 6.48 (1H, d,  $\underline{J}$  1Hz, Ar-H), 6.40 (1H, d,  $\underline{J}$  1Hz, Ar-H), 3.95 (3H, s, OMe), 3.82 (3H, s, OMe) p.p.m; m/z 224 ( $\text{M}^+$ ).

[ $4,\alpha,\alpha,\alpha\text{-}^2\text{H}_4$ ]-3,4-Dihydro-6,8-dimethoxy-3-methylisocoumarin (127)

[ $4,\alpha,\alpha,\alpha\text{-}^2\text{H}_4$ ]-6,8-Dimethoxy-3-methylisocoumarin (770 mg) was hydrogenated by the same method described for the unsubstituted compound. The product was crystallised from ether/hexane (640 mg, 82%), same data as unsubstituted compound;  $\delta$  ( $\text{CDCl}_3$ ) 6.41 (1H, s, Ar-H), 6.31 (1H, d,  $\underline{J}$  1Hz, Ar-H), 4.47 (1H, brs, 3-H), 3.88 (3H, s, OMe), 3.82 (3H, s, OMe), 2.77 (1H, brs, 4-H) p.p.m; m/z 226 ( $\text{M}^+$ ).

[ $3,4\text{-}^2\text{H}_2$ ]-3,4-Dihydro-6,8-dimethoxy-3-methylisocoumarin (123)

Deuterium gas (99.5%) was bubbled through a solution of 6,8-dimethoxy-3-methylisocoumarin (420 mg) in ethyl acetate (60 ml) in the presence of a 10% palladium on charcoal catalyst. The mixture was stirred at room temperature for 16 h, then filtered through a pad of Celite and the catalyst washed with hot ethyl acetate (20 ml). The organic solution was evaporated to give the required product which was recrystallised from ether/hexane (360 mg, 85%), same data as for

unsubstituted compound;  $\delta$   $[(\text{CD}_3)_2\text{CO}]$  6.41 (1H, d,  $J$  2Hz, Ar-H), 6.32 (1H, brs, Ar-H), 4.53 (0.5H, m, 3-H), 3.90 (3H, s, OMe), 3.84 (3H, s, OMe), 2.82 (1.5H, m, 4-H), 1.45 (2.5H, m,  $-\text{CH}_x\text{D}_y$ ) p.p.m; m/z 225, 224, 178.

### 3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin

To a solution of 3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin (140 mg) in dry dichloromethane (10 ml) was cooled to  $-70^\circ\text{C}$  and boron tribromide (1 ml) was added under nitrogen. The mixture was allowed to warm to room temperature over 16 h. The solution was then diluted with ether (20 ml) and washed with water (3 x 20 ml). The ethereal solution was dried and evaporated to give a solid which was recrystallised from acetone/hexane (75 mg, 61%), m.p.  $194-195^\circ\text{C}$ , lit.<sup>149</sup>,  $214-215^\circ\text{C}$ ;  $\nu_{\text{max}}$  3215 br, 1650 s, 1630 s  $\text{cm}^{-1}$ ;  $^1\text{H}$  200 MHz  $[(\text{CD}_3)_2\text{CO}]$  6.25 (2H, s, Ar-H), 4.67 (1H, m,  $\text{CH}-\text{CH}_3$ ), 2.87 (2H, m,  $\text{CH}_2$ ), 1.43 (3H, d,  $J$  6Hz,  $\text{CH}_3$ ) p.p.m.,  $\delta_{\text{C}}$  200 MHz  $[(\text{CD}_3)_2\text{CO}]$  170.25 (s, C=O), 164.89 (s, C-6 and C-8), 143.15 (s, C-4a and C-8a), 107.27 (d, C-7), 101.65 (d, C-5), 76.22 (d, C-3), 34.95 (t, C-4) 20.75 (q,  $\text{CH}_3$ ) p.p.m; m/z 194 ( $\text{M}^+$ ).

[4 $\alpha,\alpha,\alpha$ - $^2\text{H}_5$ ]-3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin (107)

[4 $\alpha,\alpha,\alpha$ - $^2\text{H}_4$ ]-3,4-Dihydro-6,8-dimethoxy-3-methylisocoumarin (300 mg) was demethylated by the foregoing method. The product was triturated with dichloromethane to remove any coloured impurities and recrystallised from acetone/hexane (220 mg, 83%). Same data as above;  $\delta_{\text{H}}$  200 MHz [(CD<sub>3</sub>)<sub>2</sub>CO] 11.34 (1H, s, OH), 6.33 (2H, m, 2 x Ar-H), 4.72 (1H, s, 4-H), 2.96 (1H, d,  $\underline{J}$  1Hz, 3-H) p.p.m;  $\delta_{\text{C}}$  200 MHz [(CD<sub>3</sub>)<sub>2</sub>CO] 170.66 (s, C=O), 165.16, 165.01 (s, C-6 and C-8), 148.10 (s, C-4a and C-8a), 107.40 (d, C-7), 101.70 (d, C-5), 76.11 (d, C-3), 34.50 (t, C-4), 20.70 (CD<sub>3</sub>) p.p.m; 198 (M<sup>+</sup>).

[3,4- $^2\text{H}_2$ ]-3,4-Dihydro-6,8-dihydroxy-3-methylisocoumarin (108)

[3,4- $^2\text{H}_2$ ]-3,4-Dihydro-6,8-dimethoxy-3-methylisocoumarin (300 mg) was demethylated by the foregoing method. The brown product was boiled with a solution of animal charcoal ethyl acetate for 10 min, then filtered through a pad of Celite and washed with hot ethyl acetate. The organic solvent was evaporated to give a less coloured product which was recrystallised from acetone/hexane (240 mg, 91%). Data identical with unlabelled compound  $\delta_{\text{H}}$  200 MHz [(CD<sub>3</sub>)<sub>2</sub>CO] 6.29 (2H, s, 2 x Ar-H), 2.88 (1H, brs, 4-H), 1.40 (2H, br.s, CH<sub>2</sub>D) p.p.m;  $\delta_{\text{C}}$  200 MHz [(CD<sub>3</sub>)<sub>2</sub>CO] 170.66 (s, C=O), 165.12 (s, C-6 and C-8), 143.07 (s, C-4a and C-8a), 107.39 (d, C-5), 101.96 (d, C-7), 76.25 (d, C-3), 34.80 (d, C-4), 20.61 (d, -CH<sub>X</sub>D<sub>Y</sub>) p.p.m; m/z 198-197, 196, 195, 194.

2-Bromo-3,4,5-trimethoxytoluene (138)

3,4,5-Trimethoxytoluene (10 g) was brominated by the same method used for the di-methoxy compound (118), giving a brown oil (13.9 g, 96%). T.l.c. showed 3 compounds to be present which were separated by column chromatography using a 3% ether/petroleum ether (b.p. 40-60°C) elution system to give the required compound as a clear oil which slowly crystallised on standing (8.2 g, 57%), m.p. 28-30°C (Found: C, 45.73; H, 4.91; Br, 30.45%,  $C_{10}H_{13}O_3Br$  requires C, 45.90; H, 4.98; Br, 30.60%);  $\nu_{\max}$  2920, 1560, 1485  $cm^{-1}$ ;  $\delta$  ( $CDCl_3$ ) 6.61 (1H, s, Ar-H), 3.88 (3H, s, OMe), 3.85 (3H, s, OMe), 3.82 (3H, s, OMe), 2.39 (3H, s,  $CH_3$ ) p.p.m; m/z 262, 260 ( $M^+$ ).

and :-

2,6-Dibromo-3,4,5-trimethoxytoluene (139) (2.0 g, 18%), (Found: C, 35.26, H, 3.27; Br, 46.83%,  $C_{10}H_{12}O_3Br_2$  requires C, 35.3; H, 3.5; Br, 47.0%) ;  $\delta$  ( $CDCl_3$ ) 3.88 (3H, s, OMe), 3.85 (3H, s, OMe), 3.82 (3H, s, OMe), 2.59 (3H, s,  $CH_3$ ) p.p.m; m/z 342, 340, 338. Starting material (1.9 g, 19%).

2,3,4-Trimethoxy-6-methylbenzoic acid. (140)

2-Bromo-3,4,5-trimethoxytoluene (1 g) was treated with *n*-butyllithium (2.24 ml; 1.6M in hexane) and the anion quenched with carbon dioxide in the same manner as for the di-methoxy compound (119). The required compound was isolated as a yellow oil which crystallised on standing. The yellow solid was recrystallised

from dichloromethane/hexane as white needles (0.57 g, 66%), m.p. 88-89°C. (Found: C, 58.25; H, 6.10%;  $C_{11}H_{14}O_5$  requires C, 58.14; H, 6.19%);  $\nu_{\max}$  2950 br, 1690 s, 1600  $cm^{-1}$ ;  $\delta$  ( $CDCl_3$ ) 6.55 (1H, s, Ar-H), 3.95 (3H, s, OMe), 3.87 (3H, s, OMe), 3.80 (3H, s, OMe), 2.45 (3H, s,  $CH_3$ ) p.p.m; m/z 226 ( $M^+$ ).

3,4,5-Trimethoxyhomophthalic acid (141)

2,3,4-Trimethoxy-6-methylbenzoic acid (0.4 g) was reacted with lithium diisopropylamine and the anion produced treated with dimethylcarbonate using the same procedure as for the di-methoxy-compound (115). The product was isolated as a brown oily foam which was crystallised from dichloromethane/hexane followed by recrystallisation from ethylacetate/hexane to give pale orange crystals (0.35 g, 83%), m.p. 132-133°C. (Found: C, 52.77; H, 5.04%,  $C_{12}H_{14}O_7$  requires C, 53.30; H, 5.18%);  $\nu_{\max}$  2460 br, 1720 s, 1690, 1590 s, 1565  $cm^{-1}$ ;  $\delta_H$  [ $(CD_3)_2CO$ ] 6.84 (1H, s, Ar-H), 3.90 (3H, s, OMe), 3.87 (3H, s, OMe), 3.80 (3H, s, OMe), 3.75 (2H, s,  $CH_2-CO_2H$ ) p.p.m; m/z 270 ( $M^+$ )

Attempted preparation of 2,3,4-trimethoxy-6-(2-oxopropyl)benzoic acid

3,4,5-Trimethoxyhomophthalic acid (0.57 g) was treated with acetic anhydride in pyridine in the same manner as the dimethoxy derivative (116). The compound isolated was shown to be starting material

3,4,5-Trimethoxyhomophthalic anhydride (142)

3,4,5-Trimethoxyhomophthalic acid (0.5 g) was heated at reflux with an excess of acetic anhydride for 0.5 h. The excess acetic anhydride was removed by evaporation leaving a yellow solid which was recrystallised from dichloromethane/hexane (0.28 g, 60%), m.p. 186-188°C;  $\nu_{\max}$  1780, 1770, 1750 s, 1600 s  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  200 MHz  $[(\text{CD}_3)_2\text{CO}]$  6.90 (1H, s, ArH), 4.10 (2H, s,  $\text{CH}_2$ ), 3.90 (3H, s, OMe), 3.84 (3H, s, OMe), 3.78 (3H, s, OMe) p.p.m;  $\delta_{\text{C}}$  200 MHz  $[(\text{CD}_3)_2\text{CO}]$  166.31 (s, C-1), 160.05 (s, C-3), 107.25 (d, C-5), 61.90 (q,  $\text{OCH}_3$ ), 61.16 (q,  $\text{OCH}_3$ ), 56.74 (q,  $\text{OCH}_3$ ), 35.33 (t, C-4) p.p.m; m/z 252 ( $\text{M}^+$ ).

Attempted preparation of 2,3,4-trimethoxy-6-(2-oxopropyl) benzoic acid

2,3,4-Trimethoxyhomophthalic anhydride (150 mg) was treated with acetic anhydride and pyridine in the same manner as for 2,4-dimethoxyhomophthalic acid (116) to give 2,3,4-trimethoxyhomophthalic acid (120 mg).

Attempted preparation of 4-carboxy-6,7,8-trimethoxy-3-methylisocoumarin<sup>94</sup>

Acetic anhydride (0.08 ml) and pyridine (0.106 ml) were added to a solution of 3,4,5-trimethoxyhomophthalic anhydride (150 mg) in dry THF (16 ml). The solution was stirred at room temperature for 40 min, then more acetic anhydride (0.106 ml) was added and the solution heated at gentle reflux for 1 h. After cooling the solution was evaporated and the residue dissolved in dilute aqueous sodium hydrogencarbonate (10%).



The basic solution was washed with ether (2 x 10 ml) followed by acidification with dilute hydrochloric acid and extraction with ethyl acetate (3 x 15 ml). The combined extracts were evaporated to give a yellow solid (119 mg) which on analysis was found to be starting material.

Attempted preparation of 4-acetyl-5-6-7-trimethoxyhomophthalic anhydride<sup>94</sup>

3,4,5-Trimethoxyhomophthalic acid (0.5 g) was added in small portions during 15 min to a mixture of acetic anhydride (0.78 ml) and pyridine (0.2 ml) with stirring. A second portion of acetic anhydride (0.2 ml) was then added and stirring continued. The acid slowly dissolved to form a yellow coloured solution, and after a few minutes a pale yellow solid precipitated. Dry ether (1.6 ml) was added to facilitate the stirring. After 1.5 h the solid precipitate was filtered, washed with ether and dried to give 4-isopropenylacetate-5,6,7-trimethoxyhomophthalic anhydride (149) (330 mg; 50%), m.p. 124-126°C,  $\delta_{\text{H}}$  200 MHz  $[(\text{CD}_3)_2\text{CO}]$  7.29 (1H, s, Ar-H), 4.00 (3H, s, OMe), 3.90 (3H, s, OMe), 3.85 (3H, s, OMe), 2.46 (3H, s,  $\text{OC}-\overset{\text{O}}{\parallel}\text{CH}_3$ ), 2.33 (3H, s,  $\text{C}=\text{C}-\text{CH}_3$ ) p.p.m;  $\delta_{\text{C}}$  200 MHz  $[(\text{CD}_3)_2\text{CO}]$  167.67 (s,  $\text{COCH}_3$ ), 162.84 (s, C=O), 161.53 (s, C-9), 159.27 (s, C=O), 158.73 (s, C-6), 156.19 (s, C-8), 144.04 (s, C-4a), 130.17 (s, C-7) 114.92 (s, C-8a), 110.39 (s, C-4), 107.16 (d, C-5), 62.11 (q,  $\text{OCH}_3$ ), 61.22 (q,  $\text{OCH}_3$ ), 56.73 (q,  $\text{OCH}_3$ ), 21.38 (q,  $\text{COCH}_3$ ), 21.14 (q,  $\text{C}=\text{C}-\text{CH}_3$ ) p.p.m.

2,4-Dimethoxybenzoylchloride

Excess thionyl chloride was added to 2,4-dimethoxybenzoic acid (9.1 g) until it was in solution. The mixture was then refluxed for 3 h and the excess thionyl chloride distilled off to give the product (10.8 g, 100%).

Attempted Preparation of 2,4-dimethoxy-N-methylbenzamide

25% Methylamine solution (1.66 ml) was added dropwise to 2,4-dimethoxybenzoylchloride (made from the previous reaction) at 0°C. Excess methylamine was added until the evolution of hydrogen chloride had stopped. The solution was then filtered and the solid precipitate heated with aqueous sodium hydrogencarbonate. The solid remaining was filtered, analysis showed that the compound isolated was probably the hydrochloride salt of the amide.

Attempted preparation of methyl-2,4-dimethoxybenzoate

A solution of 2,4-dimethoxybenzoic acid (1.5 g) in dry methanol (20 ml) was saturated with hydrogen chloride gas, made by dropping concentrated sulphuric acid onto sodium chloride. The mixture was heated at reflux for 4 h. After cooling the excess methanol was evaporated to half volume and sodium hydrogencarbonate solution added. The aqueous layer was extracted with ethyl acetate, dried and evaporated to give methyl 2,4-dihydroxybenzoate (1.06 g, 76%), m.p. 98-101°C;  $\nu_{\max}$  3300-2810, 1725, 1620  $\text{cm}^{-1}$ ;  $\delta[(\text{CD}_3)_2\text{CO}]$  7.80 (1H, d,  $J$  7Hz, 6-H), 6.53 (1H, d,  $J$  7Hz, 5-H), 6.48 (1H, s, 3-H), 3.85 (3H, s, COOMe) p.p.m; m/z 168 ( $\text{M}^+$ ).

Methyl 2,4-dihydroxybenzoate (157)

A solution of 2,4-dihydroxybenzoic acid (1 g) was esterified with excess methanol saturated with hydrogen chloride by the foregoing method to give the required product (0.9 g; 83%). Same data as previous page.

Methyl 2,4-dimethoxybenzoate (158)

Methyl 2,4-dihydroxybenzoate (0.5 g) in dry acetone (30 ml) with dimethyl sulphate (0.655 ml) and anhydrous potassium carbonate (1.65 g) was heated at reflux for 4 h with stirring. The reaction mixture was allowed to cool, filtered and the potassium carbonate washed with acetone (25 ml). The combined acetone solutions were evaporated to give the required product (0.54 g, 91%),  $\delta[(\text{CD}_3)_2\text{CO}]$  7.85 (1H, d,  $\underline{J}$  7Hz, 6-H), 6.70 (1H, s, 3-H), 6.65 (1H, d,  $\underline{J}$  7Hz, 5-H), 3.95 (6H, s, 2 x OMe), 3.89 (3H, s, COOMe);  $m/z$  196 ( $\text{M}^+$ ).

Attempted preparation of N-methyl-2,4-dimethoxybenzamide.

Methyl 2,4-dimethoxybenzoate (0.56 g) was treated cautiously with excess methylamine (3 ml) and the two warmed together for 1 h. Work-up revealed only starting material to be present.

Methyl 2,3,4-trimethoxybenzoate (160)

2,3,4-Trimethoxybenzoic acid (5 g) was esterified with excess methanol saturated with hydrogen chloride by the same method used for the di-methoxy acid (156). Work-up yielded the required product (4.96 g) 93%).  $\delta[(\text{CD}_3)_2\text{CO}]$  7.50 (1H, d,  $\underline{J}$  8Hz, Ar-H), 6.34 (1H, d,  $\underline{J}$  9Hz,

Ar-H), 3.97 (3H, s, COOMe), 3.87 (3H, s, OMe), 3.81 (3H, s, OMe), 3.78 (3H, s, OMe) p.p.m;  $m/z$  226 ( $M^+$ ).

N-Methyl-2,3,4-trimethoxybenzamide (151)

A 30% methylamine solution in water (27 ml) was added slowly to methyl 2,3,4-trimethoxybenzoate (5.66 g). The ester was seen to dissolve, then a white precipitate started to form. The mixture was stirred at room temperature for 20 min by which time the solution was now clear. The aqueous solution was extracted with dichloromethane, dried and evaporated to give a yellow oil (4.06 g, 76%), (128°C /0.75 mmHg);  $\nu_{\max}$  3400 s, 1650, 1600, 1550  $\text{cm}^{-1}$ ;  $\delta$  [(CD<sub>3</sub>)<sub>2</sub>CO] 7.64 (1H, d,  $J$  6Hz, Ar-H), 6.87 (1H, d,  $J$  9Hz, Ar-H), 3.92 (3H, s, OMe), 3.88 (3H, s, OMe), 3.82 (3H, s, OMe), 2.97 (3H, s, N-CH<sub>3</sub>) p.p.m;  $m/z$  225 ( $M^+$ )

Attempted preparation of 3,4-dihydro-6,7,8-trimethoxy-3-methylisocoumarin<sup>98</sup>

To a well stirred solution of N-methyl-2,3,4-trimethoxybenzamide (1 g) in THF (17 ml) was added at room temperature, *n*-butyllithium (4 ml, 1.6M in hexane) over 10 min and under nitrogen. The mixture was then refluxed for a further 40 min after which a solution of propylene oxide (1.55 ml) in 5.5 ml of ether was added over 10 min and at 0°C. The resulting solution was stirred at 0°C for 2 h and for an additional 1 h at room temperature. The reaction mixture was decomposed with water and the crude residue hydrolysed with ethanolic potassium hydroxide (10%, 22 ml) by stirring for 14 h at room temperature. After the usual work up<sup>99</sup> the only product isolated was shown to be starting material.

Attempted preparation of ethyl 2,3,4-trimethoxy-6-methylbenzoate

A solution of 2,3,4-trimethoxy-6-methylbenzoic acid (0.36 g) in dry ethanol (10 ml) and a few drops of concentrated sulphuric acid, was heated at reflux for 4 h. After cooling the mixture was evaporated to half volume and water (15 ml) added and a little solid sodium hydrogencarbonate to neutralise any remaining acid. The ethanolic solution was evaporated and the aqueous layer extracted with ethyl acetate, dried and evaporated to give a yellow oil which was shown to be virtually all starting material.

Ethyl 2,3,4-trimethoxy-6-methylbenzoate<sup>108</sup> (170)

To a stirred solution of 2,3,4-trimethoxy-6-methylbenzoic acid (0.5 g) in anhydrous dichloromethane (2.2 ml) was added 4-dimethylaminopyridine (11 mg) and ethanol (0.51 ml). The reaction mixture was then cooled to 0°C and dicyclohexylcarbodiimide (0.5 g) was added. The mixture was stirred at this temperature for 5 min and then at 20°C for 3 h. The precipitated urea was filtered off and the filtrate evaporated. The remaining residue was taken up in dichloromethane and if necessary filtered free of any further precipitated urea. The dichloromethane solution was then washed twice with dilute hydrochloric acid and then a saturated sodium hydrogencarbonate solution. The organics were then dried and evaporated to give the required compound which was recrystallised from acetone (0.34 g, 61%),  $\delta$  (CDCl<sub>3</sub>) 6.51 (1H, s, Ar-H), 4.38 (2H, q,  $J$  6Hz, OCH<sub>2</sub>-CH<sub>3</sub>), 3.90 (3H, s, OMe), 3.86 (3H, s, OMe), 3.85 (3H, s, OMe), 2.30 (3H, s, CH<sub>3</sub>), 1.40 (3H, t,  $J$  6Hz, OCH<sub>2</sub>CH<sub>3</sub>) p.p.m; m/z 254 (M<sup>+</sup>).

2-Ethoxycarbonyl-3,4,5-trimethoxybenzyl lithium<sup>102</sup> (171)

To dry THF (9 ml) containing a small crystal of triphenylmethane under nitrogen was added dropwise, n-butyllithium (1.6M in hexane) until a few drops of persistent pink colour indicated complete dryness. Diisopropylamine (0.3 ml) and n-butyllithium (1.05 ml, 1.6M in hexane) were added and the mixture stirred for 15 min at room temperature. After cooling to  $-78^{\circ}\text{C}$ , ethyl 2,3,4-trimethoxy-6-methylbenzoate (340 mg) in dry THF (4.5 ml) was added dropwise to the mixture. An orange-red metalation colour was observed indicating anion formation. The solution was stirred at  $-78^{\circ}\text{C}$  for 30 min before use.

Attempted preparation of 3,4-dihydro-6,7,8-trimethoxy-3,4-dihydroisocoumarin<sup>102</sup>

A solution of the anion was prepared as above in a separate round-bottomed flask. This was added gradually to a solution of acetaldehyde (0.39 ml, 303 mg) in THF (1.5 ml) also at  $-78^{\circ}\text{C}$  in the main body of the flask. The colour of each portion of the anion solution was discharged rapidly. After addition was complete (10 min) the solution was allowed to warm up to room temperature over 2 h with continuous stirring. Ethanol (6 ml) was then added and the reaction mixture poured onto an excess of dilute sulphuric acid. The aqueous layer was extracted twice with ether and twice with ethyl acetate. The organic layer was then washed with saturated sodium hydrogencarbonate followed by brine. Drying and evaporation yielded a yellow oil which on analysis was shown to be starting material.

Attempted preparation of 2,3,4-trimethoxy-6-bromomethylbenzoic acid<sup>109</sup>

To a solution of 2,3,4-trimethoxy-6-methylbenzoic acid (0.13 g) in carbon tetrachloride (9.75 ml) was added N-bromosuccinimide (0.21 g) and a small spatula of recrystallised azoisobutyronitrile. The solution was heated at reflux for 30 min and also irradiated with a desk lamp (150 watt). The reaction was seen to be completed when the succinimide floated on top of the reaction mixture. The succinimide was filtered off and the remaining solution washed with water (10 ml) and brine (2 x 10 ml), dried and evaporated to give a yellow crystalline solid. T.l.c. analysis of the crude product showed at least six brominated products to be present including both allylic and aromatic brominated compounds.

Attempted preparation of 1,2,3-trimethoxybenzene.

A solution of 1,2,3-trihydroxybenzene (25 g) in dry acetone (300 ml) was methylated in the usual manner. 1,2,3-Trimethoxybenzene was isolated as an oil (26.7 g, 80%), (b.p. 84°C/1.3 mmHg).

Attempted preparation of 5,6,7-trimethoxyindanone

1,2,3-Trimethoxybenzene (1 g) was heated to 150°C with aluminium trichloride (3.7 g) and  $\alpha$ -bromopropionyl bromide (1.28 g) for 4 h. The solid produced was cooled in ice and dilute hydrochloric acid added carefully until no more hydrogen chloride gas was evolved. The crude product was extracted with ethyl acetate, dried and evaporated to yield a brown solid which was purified by Kuglerohr

distillation. Analysis of the solid showed it to be 2,3,4-trihydroxyphenylethylketone propiophenone (0.1 g, 7.9%);  $\delta$  ( $\text{CDCl}_3$ ) 7.37 (1H, d,  $\underline{J}$  9Hz, Ar-H), 6.46 (1H, d,  $\underline{J}$  9Hz, Ar-H), 3.00 (2H, q,  $\underline{J}$  7Hz,  $\text{COCH}_2\text{CH}_3$ ), 1.18 (3H, t,  $\underline{J}$  7Hz,  $\text{COCH}_2\text{CH}_3$ ) p.p.m.

### 3-Bromo-4-hydroxy-5-methoxybenzaldehyde (196)

Vanillin (100 g) was dissolved in glacial acetic acid (180 ml) and the solution cooled to  $0^\circ\text{C}$ . Bromine (34.5 ml) in glacial acetic acid (30 ml) was added with rapid stirring and stirring continued at  $15^\circ\text{C}$  for a further 15 min. The resulting precipitate was washed with water, filtered and air dried. The solid was recrystallised from ethanol as white needles (123 g, 81%), m.p.  $162.5\text{--}163.5^\circ\text{C}$  (lit.<sup>150</sup>  $163\text{--}164^\circ\text{C}$ );  $\nu_{\text{max}}$  3350 br,  $1675\text{ s cm}^{-1}$ ;  $\delta[(\text{CD}_3)_2\text{CO}]$  9.80 (1H, s, CHO), 7.70 (1H, d,  $\underline{J}$  2Hz, Ar-H), 7.47 (1H, d,  $\underline{J}$  2Hz, Ar-H), 3.95 (3H, s, OMe), p.p.m;  $m/z$  232, 230 ( $\text{M}^+$ ).

### 2,5-Dihydroxy-3-methoxybromobenzene (197)

A solution of 3-bromo-4-hydroxy-5-methoxybenzaldehyde (50 g) in potassium hydroxide (400 ml, 1M) was cooled to  $0^\circ\text{C}$ . To this, a solution of hydrogen peroxide (53.3 ml, 100 vol) in water (325 ml) was added dropwise over 3 h. The mixture was stirred for a further 1 h by which time a purple solid had precipitated from solution. The precipitate was filtered, washed with water and air dried. The



product was recrystallised from water (29 g, 62%), m.p. 140-141°C (lit.<sup>150</sup> 141°C);  $\nu_{\max}$  3600-3000 br, 1620 s, 1595 s  $\text{cm}^{-1}$ ;  
 $\delta[(\text{CD}_3)_2\text{CO}]$  6.61 (1H, d,  $\underline{J}$  3Hz, Ar-H), 6.51 (1H, d,  $\underline{J}$  3Hz, Ar-H),  
 3.80 (3H, s, OMe) p.p.m; m/z 220, 218 ( $\text{M}^+$ ).

### 2,3,5-Trimethoxybromobenzene (198)

A solution of 2,5-dihydroxy-3-methylbromobenzene (54 g) in dry acetone (600 ml) with dimethylsulphate (55 ml) and anhydrous potassium carbonate (138 g) was heated at reflux for 4 h with mechanical stirring. The reaction mixture was allowed to cool, filtered and the potassium carbonate washed with acetone (100 ml). The combined acetone solutions were evaporated and the residue refluxed with sodium hydroxide solution (50 ml, 30%) for 1 h to ensure all the dimethylsulphate had been destroyed. The product was extracted with ether (100 ml) and the ether layer washed with water (100 ml), sodium hydroxide (50 ml, 10%) and water (50 ml), dried and evaporated. The required compound was isolated as a pale yellow oil (49.4 g, 83%), (b.p. 78°C/0.05 mmHg) which crystallised on standing m.p. 26.5 - 27°C (lit.<sup>150</sup> 37-38°C)  
 $\delta[(\text{CD}_3)_2\text{CO}]$  6.71 (1H, d,  $\underline{J}$  3Hz, Ar-H), 6.63 (1H, d,  $\underline{J}$  3Hz, Ar-H),  
 3.88 (3H, s, OMe), 3.80 (3H, s, OMe), 3.76 (3H, s, OMe) p.p.m;  
 m/z 248, 246.

2,3,5-Trimethoxybenzoic acid (199)

Ether (75 ml) containing 2,3,5-trimethoxybenzoic acid (13.6 g) was cooled to  $-70^{\circ}\text{C}$  and a solution of *n*-butyllithium (35 ml, 1.6M in hexane) was added with stirring. Stirring was continued for 1 h after which the mixture was poured onto crushed dry-ice (149 g). This was allowed to warm up to room temperature and then water (37 ml) was added. The ether layer was separated and extracted with water (37 ml). The aqueous extracts were acidified and cooled. After standing at  $0^{\circ}\text{C}$  for 2 h a precipitate was formed which was collected, washed with water and air dried. The product was recrystallised from hexane (7.34 g, 64%), m.p.  $98-100^{\circ}\text{C}$  (lit.<sup>151</sup>  $99.5-100.5^{\circ}\text{C}$ );  $\nu_{\text{max}}$  3000-2800 br,  $1750\text{ cm}^{-1}$ ;  $\delta[(\text{CD}_3)_2\text{CO}]$  6.91 (1H, d,  $J$  3Hz, Ar-H), 6.80 (1H, d,  $J$  3Hz, Ar-H), 3.88 (6H, s, 2xOMe), 3.79 (3H, s, OMe) p.p.m;  $m/z$  212 ( $\text{M}^+$ )

2,3,5-Trihydroxybenzoic acid (200)

To a solution of 2,3,5-trimethoxybenzoic acid (3.45 g) in chlorobenzene (30 ml) was added powdered anhydrous aluminium chloride (10 g) with stirring and the mixture refluxed for 45 min. The reaction mixture was allowed to cool then poured onto ice (30 g) and the flask washed with water (100 ml). The two layers were filtered through Celite and the aqueous layer separated and washed with ether (100 ml). The clear brown aqueous layer was acidified with concentrated hydrochloric acid (8 ml, 36%) from which a bright yellow solid precipitated. This was collected after standing at  $0^{\circ}\text{C}$  for 2 h

and air dried to give (2.05 g, 71%). A small portion was recrystallised from glacial acetic acid m.p. 233-234°C (lit.<sup>151</sup> 234.5 - 235.5°C);  $\nu_{\max}$  3600-3100 br, 1670 m, 1630, 1605  $\text{cm}^{-1}$ ;  $\delta[(\text{CD}_3)_2\text{CO}]$  6.83 (1H, d,  $\underline{J}$  4Hz, Ar-H), 6.65 (1H, d,  $\underline{J}$  4Hz, Ar-H) p.p.m; m/z 170 ( $\text{M}^+$ ).

Methyl 2,3,5-trihydroxybenzoate (201)

A solution of 2,3,5-trihydroxybenzoic acid (1.5 g) in dry methanol (22 ml) was saturated with hydrogen chloride and heated at reflux for 4 h. After cooling, the mixture was evaporated to leave a brown gum which was triturated with petroleum ether (b.p. 60-80°C) to give a yellow crystalline solid which was filtered and dried. The product was recrystallised from ethyl acetate/hexane (0.84 g, 51%), m.p. 61-63°C (lit.<sup>120</sup> 63-64°C);  $\nu_{\max}$  3600-3000 br, 1680 m, 1620  $\text{cm}^{-1}$ ;  $\delta[(\text{CD}_3)_2\text{CO}]$  6.81 (1H, d,  $\underline{J}$  3Hz, Ar-H), 6.71 (1H, d,  $\underline{J}$  3Hz, Ar-H), 3.91 (3H, s,  $\text{COOCH}_3$ ) p.p.m; m/z 184 ( $\text{M}^+$ ).

Methyl 2,3,5-tribenzyloxybenzoate (202)

To a solution of methyl 2,3,5-trihydroxybenzoate (0.9 g) in dry butanone (22.5 ml) was added benzyl bromide (3.6 ml), anhydrous potassium carbonate (4.5 g) and a few crystals of potassium iodide. The mixture was stirred and refluxed overnight. On cooling, the solution was filtered to remove the potassium carbonate which was washed with acetone.

The combined washings were evaporated under vacuum and the excess benzyl bromide removed by distillation at high vacuum or by washing successively with ammonia solution. The residue was crystallised from diisopropylether (0.81 g, 37%), m.p. 79-81°C (lit.<sup>120</sup> 100-102°C);  $\nu_{\max}$  3500-3200, 3100-2840, 1690 s, 1605 s  $\text{cm}^{-1}$ ;  $\delta[(\text{CD}_3)_2\text{CO}]$  7.40 (1H, m,  $3\times\text{OCH}_2\text{Ph}$ ), 6.97 (1H, d,  $\underline{J}$  3Hz, Ar-H), 6.86 (1H, d,  $\underline{J}$  3Hz, Ar-H), 5.15 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 5.07 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 5.00 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 3.76 (3H, s,  $\text{COOCH}_3$ ) p.p.m; m/z 454 ( $\text{M}^+$ )

Ethylphosphonic acid bis(dimethylamide) (186)

To a stirred solution of ethylphosphonic dichloride (5 g) in ether (125 ml) at 0°C and under nitrogen was added dimethylamine (12.5 ml). The resulting solution was stirred at 0°C for 1 h and at room temperature for a further 3 h. The precipitate of dimethylamine hydrochloride was removed by filtration and the filtrate evaporated under vacuum to give a yellow oil. Distillation of the residue afforded a colourless oil (3.29 g, 60%), b.p. 112°C/3.1 mmHg (lit.<sup>152</sup> b.p. 142°/31 mmHg);  $\delta(\text{CDCl}_3)$  2.67 (12H, d,  $^3\text{J}_{\text{PH}}$  9Hz,  $2 \times \text{N}(\text{CH}_3)_2$ ), 1.83 (2H, dq,  $\underline{J}_{\text{HH}}$  6Hz and  $^2\text{J}_{\text{PH}}$  14Hz,  $\text{CH}_2\text{-CH}_3$ ), 1.12 (3H, dt,  $\underline{J}_{\text{HH}}$  6Hz and  $^2\text{J}_{\text{PH}}$  18Hz,  $\text{CH}_2\text{CH}_3$ ) p.p.m.

2-[1-(2,3,5-Tribenzyloxyphenyl)-1-oxopropyl]phosphonic acid  
(bisdimethylamide) (203)

A stirred solution containing ethyl phosphonic acid bis(dimethylamide) (1 g) in dry THF (15 ml) was treated while at  $-78^{\circ}\text{C}$  and under nitrogen with a solution of *n*-butyllithium (3.9 ml, 1.6M in hexane). The mixture was stirred at  $-50^{\circ}\text{C}$  for 3 h. After cooling to  $-78^{\circ}\text{C}$  a solution of methyl 2,3,5-tribenzyloxybenzoate (1.36 g) in dry THF was added. Stirring was continued for 2 h at  $-70^{\circ}\text{C}$  and 0.5 h at  $-70^{\circ}\text{C}$  to  $+25^{\circ}\text{C}$ . Water was then added and the THF removed by evaporation under nitrogen. The aqueous solution was extracted with ethyl acetate and the combined organic extracts were washed thoroughly with sodium chloride solution (1M) to remove any unreacted ethylphosphonic bis(dimethylamide). The organics were dried and evaporated to give a yellow solid which was recrystallised from diisopropylether (0.98 g, 56%), m.p.  $140-142^{\circ}\text{C}$  (lit.<sup>120</sup>  $147-149^{\circ}\text{C}$ );  $\nu_{\text{max}}$  3100-2790 s, 1670 s, 1600 s  $\text{cm}^{-1}$ ;  $\delta(\text{CDCl}_3)$  7.35 (15H, m,  $3\times\text{OCH}_2\text{Ph}$ ), 6.79 (1H, d,  $J$  3Hz, Ar-H), 6.75 (1H, d,  $J$  3Hz, Ar-H), 5.14 and 4.90 (2H, AB,  $J_{\text{AB}}$  26 Hz,  $\text{OCH}_2\text{Ph}$ ), 5.22 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 5.11 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 4.51 (1H, dq,  $J_{\text{HH}}$  7Hz and  $J_{\text{PH}}$  19Hz,  $\text{COCHCH}_3\text{P}(\text{O})(\text{NMe}_2)_2$ ), 2.48 (6H, d,  $^3J_{\text{PH}}$  10Hz,  $\text{P}(\text{O})(\text{NMe}_2)_2$ ), 2.41 (6H, d,  $^3J_{\text{PH}}$  10Hz,  $\text{P}(\text{O})(\text{NMe}_2)_2$ ) 1.28 (3H, dd,  $J_{\text{HH}}$  7Hz and  $^3J_{\text{PH}}$  18Hz,  $\text{COCHCH}_3\text{P}(\text{O})(\text{NMe}_2)_2$ ) p.p.m.

2-[1-(2,3,5-Tribenzyloxyphenyl)-1-hydroxypropyl]phosphonic acid bis(dimethylamide) (194)

To a stirred solution of  $\beta$ -ketophosphonic acid bis(dimethylamide) (0.25 g) in methanol (7.5 ml) was slowly added while at  $0^{\circ}\text{C}$ , sodium borohydride (0.25 g). Stirring was continued for 1 h, after which dilute hydrochloric acid (0.1M) was carefully added to destroy the excess sodium borohydride ensuring that the pH of the solution remained above 7.5. A colourless precipitate formed which was collected, washed with cold water and air dried. A sample was recrystallised from diisopropylether (0.21 g, 84%), m.p.  $125-128^{\circ}\text{C}$  (lit.<sup>120</sup>  $131-133^{\circ}\text{C}$  decomp.);  $\delta[(\text{CD}_3)_2\text{CO}]$  7.40 (15H, m,  $3\times\text{OCH}_2\text{Ph}$ ), 6.79 (2H, s,  $2\times\text{Ar-H}$ ), 5.20 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 5.10 (2H, s,  $\text{OCH}_2\text{Ph}$ ) 4.92 (2H, s,  $\text{OCH}_2\text{Ph}$ ), 5.23 - 4.80 (2H, m,  $\text{ArCH}_2\text{OHCHCH}_3\text{P}(\text{O})(\text{NMe}_2)_2$ ), 2.65 (6H, d,  $^3\text{J}_{\text{PH}}$  10Hz,  $\text{RP}(\text{O})\text{NMe}_2$ ), 2.53 (6H, d,  $\text{J}$  10Hz,  $\text{RP}(\text{O})\text{NMe}_2$ ), 0.83 (3H, dd,  $\text{J}_{\text{HH}}$  7Hz and  $^3\text{J}_{\text{PH}}$  17Hz,  $\text{RCHCH}_3\text{P}(\text{O})(\text{NMe}_2)_2$ ) p.p.m.

Attempted preparation of 2-[1-(2,3,5-tribenzyloxyphenyl)-1-hydroxypropyl] phosphonic acid bis(dimethylamide)

A solution of 2-[1-(2,3,5-tribenzyloxyphenyl)-1-hydroxypropyl] phosphonic acid bis(dimethylamide) (100 mg) in methanol (15 ml) containing 10% palladium on charcoal was hydrogenated at atmospheric pressure for 1 h. The catalyst was removed by filtration through Celite and the organics evaporated. Spectroscopic analysis of the isolated product showed that the compound had been entirely degraded appearing as a black solid.

Vanillin allyl ether<sup>128</sup> (209)

Vanillin (20 g) was dissolved in a solution of sodium hydroxide (200 ml, 0.82 M). To this was added allyl bromide (15.9 g, 11.37 ml) and the mixture refluxed for 2 h. The solution was allowed to cool, then extracted with ether (2 x 200 ml), dried and evaporated. Any excess allyl bromide was distilled off (b.p. 70-71°C/mmHg) leaving behind a yellow oil (13.45 g). T.l.c. analysis of the crude product (50% ether/petroleum ether (b.p. 40-60°C) showed five compounds to be present. The crude oil was purified using column chromatography, eluting with a 5% ether/petroleum ether (b.p. 40-60°C) solvent system to give the required compound as the major product (8.49 g, 34%). (See chapter 4 for discussion of products). The major product was found at,  $R_f$  0.4:  $\delta_H$  200 MHz (CDCl<sub>3</sub>) 9.68 (1H, s, CHO), 7.23 (2H, m, 3-H and 5-H), 6.79 (1H, d,  $J$  9Hz, 2-H), 5.92 (1H, X of ABX system, CH=CH<sub>2</sub>), 5.21 (2H, AB of ABX system, CH=CH<sub>2</sub>), 4.52 (2H, dt,  $J$  1.5 Hz and 5.4 Hz OCH<sub>2</sub>CH=CH<sub>2</sub>), 3.74 (3H, s, OMe) p.p.m;  $\delta_C$  200 MHz (CDCl<sub>3</sub>) 190.28 (d, CHO), 152.95 (s, C-3 or C-4), 149.36 (s, C-3 or C-4), 131.85 (d, OCH<sub>2</sub>-CH=CH<sub>2</sub>), 129.68 (s, C-1), 125.98 (d, C-6), 118.11 (t, OCH<sub>2</sub>-CH=CH<sub>2</sub>), 111.46 (d, C-2 or C-5), 108.81 (d, C-2 or C-5), 69.19 (t, OCH<sub>2</sub>-CH=CH<sub>2</sub>), 55.43 (q, OCH<sub>3</sub>) p.p.m;  $m/z$  192 (M<sup>+</sup>). Minor products at,  $R_f$  0.90:  $\delta_H$  (CDCl<sub>3</sub>) 6.62 (2H, s, 2xAr-H), 6.38-5.70 (3H, m, ArOCH<sub>2</sub>-CH=CH<sub>2</sub> and 2xArCH<sub>2</sub>-CH=CH<sub>2</sub>), 5.45-4.90 (6H, m, ArOCH<sub>2</sub>-CH=CH<sub>2</sub> and 2xArCH<sub>2</sub>-CH=CH<sub>2</sub>), 4.45 (2H, d,  $J$  6Hz, ArOCH<sub>2</sub>-CH=CH<sub>2</sub>), 3.80 (3H, s, ArOCH<sub>3</sub>), 3.40 (2H, d,  $J$  6Hz, ArCH<sub>2</sub>-CH=CH<sub>2</sub>), 3.32 (2H, d,  $J$  6Hz, ArCH<sub>2</sub>-CH=CH<sub>2</sub>) p.p.m;  $m/z$  244 (M<sup>+</sup>).

$R_f$  0.85:  $\delta_H$  ( $CDCl_3$ ) 6.80 (3H, m, 3xAr-H), 6.35-5.25 (2H, m, Ar  $OCH_2-CH=CH_2$  and  $ArCH_2-CH=CH_2$ ), 5.50-5.05 (4H, m, Ar- $OCH_2-CH=CH_2$  and  $ArCH_2-CH=CH_2$ ), 4.62 (2H, d,  $J$  4.5 Hz,  $ArOCH_2CH=CH_2$ ), 3.89 (3H, s, Ar- $OCH_3$ ), 3.38 (2H, d,  $J$  6Hz,  $ArCH_2-CH=CH_2$ ) p.p.m;  $m/z$  204 ( $M^+$ )

$R_f$  0.60:  $\delta_H$  ( $CDCl_3$ ) 9.88 (1H, s, ArCHO), 7.34 (2H, s, 2xAr-H), 6.27-5.76 (2H, m,  $ArOCH_2-CH=CH_2$  and  $ArCH_2-CH=CH_2$ ), 5.45-4.95 (4H, m,  $ArOCH_2-CH=CH_2$  and  $ArCH_2-CH=CH_2$ ), 4.59 (2H, d,  $J$  5Hz,  $ArOCH_2-CH=CH_2$ ), 3.89 (3H, s,  $ArOCH_3$ ), 3.45 (2H, d,  $J$  6Hz,  $ArCH_2-CH=CH_2$ ) p.p.m;  $m/z$  232 ( $M^+$ ).

$R_f$  0.25 :  $\delta_H$  ( $CDCl_3$ ) 9.86 (1H, d,  $J$  7Hz,  $ArCH=CH-CHO$ ), 7.45 (1H, br.d,  $J$  17Hz,  $ArCH=CH-CHO$ ), 7.13 (2H, s, 2xAr-H), 6.75-6.47 (1H, dd,  $J$  8Hz and 17Hz,  $ArCH=CH-CHO$ ), 6.35-5.90 (1H, m,  $ArOCH_2-CH=CH_2$ ), 5.55-5.25 (2H, m,  $ArOCH_2-CH=CH_2$ ), 4.67 (2H, d,  $J$  5Hz,  $ArOCH_2-CH=CH_2$ ), 3.95 (3H, s,  $ArOCH_3$ ) p.p.m;  $m/z$  218 ( $M^+$ ).

### Allyl Vanillin<sup>128</sup> (210)

Vanillin allyl ether (2 g) was dissolved in N,N-dipropylaniline (10 ml) and the mixture refluxed at a temperature  $> 200^\circ C$  for 1 h. The solution was allowed to cool and ethyl acetate (20 ml) was added, the organic solution was washed with dilute hydrochloric acid to remove any N,N-dipropylaniline. The organic layer was evaporated to leave an oil which was dissolved in a 5M sodium hydroxide solution (2 ml) and washed with light petroleum 40-60°C which removed any trace amounts of 2-methyldihydrobenzofuran derivatives that may be formed as a by-product.



The aqueous layer was carefully acidified with 5M hydrochloric acid, then extracted with ether. The extract was dried and evaporated to give a brown oil (1.82 g). T.l.c. analysis [75% ether/petroleum ether (b.p. 40-60°C)] showed several compounds to be present. Column chromatography using 5% ether/petroleum ether (b.p. 40-60°C) as eluant furnished the required compound as the major product (0.4 g, 20%), m.p. 84-85°C (lit.<sup>128</sup> 85-86°C);  $\delta$  (CDCl<sub>3</sub>) 9.78 (1H, s, CHO), 7.30 (2H, m, 3H and 5H), 6.02 (1H, m, CH<sub>2</sub>-CH=CH<sub>2</sub>), 5.10 (2H, m, CH<sub>2</sub>-CH=CH<sub>2</sub>), 4.39 (3H, s, OMe), 3.47 (2H, d,  $J$  6Hz, Ar-CH<sub>2</sub>) p.p.m; m/z 192 (M<sup>+</sup>)

5-Propenyl vanillin<sup>134</sup> (211)

Potassium hydroxide pellets (2.81 g) dissolved in methanol (3.51 g) was added to allyl vanillin (150 mg) and the mixture heated at reflux for 6 h with occasional thorough mixing. The solution was allowed to cool then acidified with concentrated hydrochloric acid. The aqueous solution was extracted with ether (3 x 20 ml), dried and evaporated. T.l.c. analysis [75% ether/petroleum ether (b.p. 40-60°C)] showed mostly one major product to be present. This was purified by column chromatography eluting with a 5% ether/petroleum ether (b.p. 40-60°C) mixture. The required compound was obtained as a white powder which was recrystallised from ether/petroleum ether (b.p. 40-60°C) to give (140 mg, 93%), m.p. 92-94°C (lit.<sup>134</sup> 98-99°C)  $\delta$  (CDCl<sub>3</sub>) 9.81 (1H, s, ArCHO), 7.50 (1H, s, 6-H), 7.30 (1H, s, 2-H), 6.60 (1H, brs, CH=CH-CH<sub>3</sub>), 6.35 (1H, m, CH=CH-CH<sub>3</sub>), 3.97 (3H, s, OMe), 1.97 (3H, d,  $J$  6Hz, CH=CH-CH<sub>3</sub>) p.p.m; m/z 192 (M<sup>+</sup>).

Attempted preparation of 3-methoxy-2,5-dihydroxyphenylpropene<sup>135</sup>

A solution of 5-propenylvanillin (60 mg) in aqueous potassium hydroxide (2.84 ml, 0.1M) was cooled to 0°C. To this solution was added dropwise hydrogen peroxide (0.076 ml, 100 vol) in water (0.46 ml). The mixture was stirred for 4 h by which time a purple solid had precipitated. This solid was filtered, washed with water and air dried. However analysis of the product showed it to be starting material.

A second approach to 3-methoxy-2,5-dihydroxyphenylpropene<sup>133</sup>

5-Propenyl vanillin (200 mg) was added to a sodium hydroxide solution (0.65 ml, 2M) and the mixture heated until all the solid material was in solution. A 6% hydrogen peroxide solution (0.93 ml) was then added dropwise over 3 h, taking care to keep the reaction temperature below 50°C. The reaction mixture was stirred for a further 1 h then worked up as in the previous method. An unidentified product was obtained.

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